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(54) Title: DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS			
(57) Abstract <p>The duplex DNA of chromosomes is replicated in a multicomponent process. A helicase unwinds the DNA, a replicase synthesizes new DNA, and primase repeatedly synthesizes new primed starts on the lagging strand. The present invention is directed to the genes from Gram positive bacterium encoding these proteins, and their characterization. Replicases are highly efficient polymerases. There are several mechanisms by which a replicase can achieve high processivity. The invention determines that the replicase of <i>Staphylococcus</i> operates as a 3 component system in which a clamp loader enzyme assembles a sliding clamp protein onto DNA. The sliding clamp then binds the DNA polymerase III holoenzyme making it highly efficient. The invention identifies two DNA polymerase III enzymes in Gram positive bacterium, each of which operate with the clamp and clamp loader, to extend a single primed site around a long (over 5kb) ssDNA template. These replication proteins can be utilized in a variety of assays to screen chemical compound libraries for an antibiotic compound.</p>			

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DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS

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respectively

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FIELD OF THE INVENTION

This invention relates to genes and proteins that replicate the
chromosome of Gram positive bacteria. These proteins can be used in drug discovery
15 to screen large libraries of chemicals for identification of compounds with antibiotic
activity.

BACKGROUND OF THE INVENTION

20 All forms of life must duplicate the genetic material to propagate the
species. The process by which the DNA in a chromosome is duplicated is called
replication. The replication process is performed by numerous proteins that
coordinate their actions to smoothly duplicate the DNA. The main protein actors are
as follows (reviewed in Kornberg, et al., DNA Replication, Second Edition, New
25 York: W.H. Freeman and Company, pp. 165-194 (1992)). A helicase uses the energy
of ATP hydrolysis to unwind the two DNA strands of the double helix. Two copies
of the DNA polymerase use each "daughter" strand as a template to convert them into
two new duplexes. The DNA polymerase acts by polymerizing the four monomer
unit building blocks of DNA (the 4 dNTPs, or deoxynucleoside triphosphates are:
30 dATP, dCTP, dGTP, dTTP). The polymerase rides along one strand of DNA using it
as a template that dictates the sequence in which the monomer blocks are to be
polymerized. Sometimes the DNA polymerase makes a mistake and includes an

incorrect nucleotide (e.g., A instead of G). A proofreading exonuclease examines the polymer as it is made and excises building blocks that have been improperly inserted in the polymer.

Duplex DNA is composed of two strands that are oriented antiparallel to one another, one being oriented 3'-5' and the other 5' to 3'. As the helicase unwinds the duplex, the DNA polymerase moves continuously forward with the helicase on one strand (called the leading strand). However, due to the fact that DNA polymerases can only extend the DNA forward from a 3' terminus, the polymerase on the other strand extends DNA in the opposite direction of DNA unwinding (called the lagging strand). This necessitates a discontinuous ratcheting motion on the lagging strand in which the DNA is made as a series of Okazaki fragments. DNA polymerases cannot initiate DNA synthesis *de novo*, but require a primed site (i.e. a short duplex region). This job is fulfilled by primase, a specialized RNA polymerase, that synthesizes short RNA primers on the lagging strand. The primed sites are extended by DNA polymerase. A single stranded DNA binding protein (SSB) is also needed; it operates on the lagging strand. The function of SSB is to coat single stranded DNA (ssDNA), thereby melting short hairpin duplexes that would otherwise impede DNA synthesis by DNA polymerase.

The replication process is best understood for the Gram negative bacterium, *Escherichia coli*, and its bacteriophages T4 and T7 (reviewed in Kelman, et al., "DNA Polymerase III Holoenzyme: Structure and Function of Chromosomal Replicating Machine," Annu. Rev. Biochem., 64:171-200 (1995); Marians, K.J., "Prokaryotic DNA Replication," Annu. Rev. Biochem., 61:673-719 (1992); McHenry, C.S., "DNA Polymerase III Holoenzyme: Components, Structure, and Mechanism of a True Replicative Complex," J. Bio. Chem., 266:19127-19130 (1991); Young et. al., "Structure and Function of the Bacteriophage T4 DNA Polymerase Holoenzyme," Am. Chem. Soc., 31:8675-8690 (1992)). The eukaryotic systems of yeast (*Saccharomyces cerevisiae*) (Morrison et. al., "A Third Essential DNA Polymerase in *S. cerevisiae*," Cell, 62:1143-51 (1990) and humans (Bambara, et al., "Reconstitution of Mammalian DNA Replication," Prog. Nuc. Acid Res., 51:93-123 (1995)) have also been characterized in some detail as has herpes virus (Boehmer, et al., "Herpes Simplex Virus DNA Replication," Annu. Rev. Biochem., 66:347-384

(1997)) and vaccinia virus (McDonald, et. al., "Characterization of a Processive Form of the Vaccinia Virus DNA Polymerase," Virology, 234:168-175 (1997)). The helicase of *E. coli* is encoded by the *dnaB* gene and is called the DnaB-helicase. In phage T4, the helicase is the product of the gene 41, and, in T7, it is the product of gene 4. Generally, the helicase contacts the DNA polymerase, in *E. coli*. This contact is necessary for the helicase to achieve the catalytic efficiency needed to replicate a chromosome (Kim, et. al., "Coupling of a Replicative Polymerase and Helicase: A tau-DnaB Interaction Mediates Rapid Replication Fork Movement," Cell, 84:643-650 (1996)). The identity of the helicase that acts at the replication fork in a eukaryotic cellular system is still not firm.

The primase of *E. coli* (product of the *dnaG* gene), phage T4 (product of gene 61), and T7 (gene 4) require the presence of their cognate helicase for activity. The primase of eukaryotes, called DNA polymerase alpha, looks and behaves differently. DNA polymerase alpha is composed of 4 subunits. The primase activity is associated with the two smaller subunits, and the largest subunit is the DNA polymerase which extends the product of the priming subunits. DNA polymerase alpha does not need a helicase for priming activity.

The chromosomal replicating DNA polymerase of all these systems, prokaryotic and eukaryotic, share the feature that they are processive, meaning they remain continuously associated with the DNA template as they link monomer units (dNTPs) together. This catalytic efficiency can be manifest *in vitro* by their ability to extend a single primer around a circular single stranded DNA (ssDNA) of over 5,000 nucleotide units in length. Chromosomal DNA polymerases will be referred to here as replicases to distinguish them from DNA polymerases that function in other DNA metabolic processes and are far less processive.

There are three types of replicases known thus far that differ in how they achieve processivity, and how their subunits are organized. These will be referred to here as Types I-III. The Type I is exemplified by the phage T5 replicase, which is composed of only one subunit yet is highly processive (Das, et al., "Mechanism of Primer-template Dependent Conversion of dNTP-dNMP by T7 DNA Polymerase," J. Biol. Chem., 255:7149-7154 (1980)). It is possible that the T5 enzyme achieves processivity by having a cavity within it for binding DNA, and that

a domain of the protein acts as a lid that opens to accept the DNA, and closes to trap the DNA inside, thereby keeping the polymerase on DNA during polymerization of dNTPs. Type II is exemplified by the replicases of phage T7, herpes simplex virus, and vaccinia virus. In these systems, the replicase is composed of two subunits, the DNA polymerase and an "accessory protein" which is needed for the polymerase to become highly efficient. It is presumed that the DNA polymerase binds the DNA in a groove and that the accessory protein forms a cap over the groove trapping the DNA inside for processive action. Type III is exemplified by the replicases of *E. coli*, phage T4, yeast, and humans in which there are three separate components, a sliding clamp protein, a clamp loader protein complex, and the DNA polymerase. In these systems, the sliding clamp protein is an oligomer in the shape of a ring. The clamp loader is a multiprotein complex which uses ATP to assemble the clamp around DNA. The DNA polymerase then binds the clamp which tethers the polymerase to DNA for high processivity. The replicase of the *E. coli* system contains a fourth component called tau that acts as a glue to hold two polymerases and one clamp loader together into one structure called Pol III*. In this application, any replicase that uses a minimum of three components (i.e. clamp, clamp loader, and DNA polymerase) will be referred to as either a type III enzyme or as a DNA polymerase III-type replicase.

The *E. coli* replicase is also called DNA polymerase III holoenzyme. The holoenzyme is a single multiprotein particle that contains all the components and therefore is composed of 10 different proteins. This holoenzyme is suborganized into four functional components called: 1) Pol III core (DNA polymerase); 2) gamma complex (clamp loader); 3) beta subunit (sliding clamp); and 4) tau (glue protein). The DNA polymerase III "core" is a tightly associated complex containing one each of the following three subunits: 1) the alpha subunit is the actual DNA polymerase (129 kDa); 2) the epsilon subunit (28 kDa) contains the proofreading 3'-5' exonuclease activity; and 3) the theta subunit has an unknown function. The gamma complex is the clamp loader and contains the following subunits: gamma, delta, delta prime, chi and psi (U.S. Patent No. 5,583,026 to O'Donnell). The beta subunit is a homodimer and forms the ring shaped sliding clamp. These components associate to form the holoenzyme and the entire holoenzyme can be assembled *in vitro* from 10 isolated pure subunits (U.S. Patent No. 5,583,026 to O'Donnell; U.S. Patent No.

5,668,004 to O'Donnell). The tau subunit, encoded by the same gene that encodes gamma (dnaX), acts as a glue to hold two cores together with one gamma complex. This subassembly is called DNA polymerase III star (Pol III*). One beta ring interacts with each core in Pol III* to form DNA polymerase III holoenzyme.

5 During replication, the two cores in the holoenzyme act coordinately to synthesize both strands of DNA in a duplex chromosome. At the replication fork, DNA polymerase III holoenzyme physically interacts with the DnaB helicase through the tau subunit to form a yet larger protein complex termed the "replisome" (Kim, et. al., "Coupling of a Replicative Polymerase and Helicase: A tau-DnaB Interaction
10 Mediates Rapid Replication Fork Movement," Cell, 84:643-650 (1996); Yuzhakov, et. al., "Replisome Assembly Reveals the Basis for Asymmetric Function in Leading and Lagging Strand Replication," Cell, 86:877-886 (1996)). The primase repeatedly contacts the helicase during replication fork movement to synthesize RNA primers on the lagging strand (Marians, K.J., "Prokaryotic DNA Replication," Annu. Rev.
15 Biochem., 61:673-719 (1992)).

 In the present invention, new genes from Gram positive bacteria (e.g., *S. aureus*) are identified. Although their homology with *E. coli* proteins is often weak, they will be assigned names based on their nearest homology to subunits in the *E. coli* system. The gene of *E. coli* replication proteins are as follows: alpha (dnaE);
20 epsilon (dnaQ); theta (holE); tau (dnaX); gamma (dnaX); delta (holA); delta prime (holB); chi (holC); psi (holD); beta (dnaN); DnaB; helicase (dnaB); and primase (dnaG).

 The dnaX gene encodes both tau and gamma. Tau is the product of the full gene. Gamma is the product of the first 2/3 of the gene; it is truncated by an
25 efficient translational frameshift that results in incorporation of one unique residue followed by a stop codon.

 Although there are many studies of replication mechanisms in eukaryotes, and the Gram negative bacterium, *E. coli* and its bacteriophages, there is very little information about how Gram positive organisms replicate. The
30 evolutionary split between Gram positive bacteria and Gram negative bacteria occurred approximately 1.2 billion years ago. The Gram positive class of bacteria includes some of the worst human pathogens such as *Staphylococcus aureus*,

Streptococcus pneumoniae, *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Mycobacterium tuberculosis* (Youmans, et. al., The Biological and Clinical Basis of Infectious Disease (1985)).

Currently, the best characterized Gram positive organism for DNA synthesis is *Bacillus subtilis*. Fractionation of *B. subtilis* has identified three DNA polymerases. Gass, et al., "Further Genetic and Enzymological Characterization of the Three *Bacillus subtilis* Deoxyribonucleic Acid Polymerases," J. Bio. Chem., 248:7688-7700 (1973); Ganesan, et. al., "DNA Replication in a Polymerase I Deficient Mutant and the Identification of DNA Polymerases II and III in *Bacillus subtilis*," Biochem. And Biophys. Res. Commun., 50:155-163 (1973)). These polymerases are thought to be analogous to the three DNA polymerases of *E. coli* (DNA polymerases I, II and III). Studies in *B. subtilis* have identified a polymerase that appears to be involved in chromosome replication and is termed Pol III (Ott, et. al., "Cloning and Characterization of the PolC Region of *Bacillus subtilis*," J. Bacteriol., 165:951-957 (1986); Barnes, et. al., "Localization of the Exonuclease and Polymerase Domains of *Bacillus subtilis* DNA Polymerase III," Gene, 111:43-49 (1992); Barnes, et. al., "The 3'-5' Exonuclease Site of DNA Polymerase III From Gram-positive Bacteria: Definition of a Novel Motif Structure," Gene" 165:45-50 (1995) or Barnes, et al., "Purification of DNA Polymerase III of Gram-positive Bacteria," Methods in Enzy., 262:35-42 (1995)). The *B. subtilis* Pol III (called PolC) is larger (about 165 kDa) than the *E. coli* alpha subunit (about 129 kDa) and exhibits 3'-5' exonuclease activity. The PolC gene encoding this Pol III shows weak homology to the genes encoding *E. coli* alpha and the *E. coli* epsilon subunit. Hence, this long form of the *B. subtilis* Pol III (herein referred to as Pol III-L) essentially comprises both the alpha and epsilon subunits of the *E. coli* core polymerase. The *S. aureus* Pol III-L has also been sequenced, expressed in *E. coli* and purified; it contains polymerase and 3'-5' exonuclease activity (Pacitti, et. al., "Characterization and Overexpression of the Gene Encoding *Staphylococcus aureus* DNA Polymerase III," Gene, 165:51-56 (1995)). Although this Pol III-L is essential to cell growth (Clements, et. al., "Inhibition of *Bacillus subtilis* Deoxyribonucleic Acid Polymerase III by Phenylhydrazinopyrimidines: Demonstration of a Drug-induced Deoxyribonucleic Acid-Enzyme Complex," J. Biol. Chem., 250:522-526 (1975);

Cozzarelli, et al., "Mutational Alteration of *Bacillus subtilis* DNA Polymerase III to Hydroxyphenylazopyrimidine Resistance: Polymerase III is Necessary for DNA Replication," Biochem. And Biophys. Res. Commun., 51:151-157 (1973); Low, et. al., "Mechanism of Inhibition of *Bacillus subtilis* DNA Polymerase III by the Arylhydrazinopyrimidine Antimicrobial Agents," Proc. Natl. Acad. Sci. USA, 71:2973-2977 (1974)), there could still be another DNA polymerase(s) that is essential to the cell, such as occurs in yeast (Morrison, et. al., "A Third Essential DNA Polymerase in *S. cerevisiae*," Cell, 62:1143-1151 (1990)).

Purification of Pol III-L from *B. subtilis* results in only this single protein without associated proteins Barnes, et. al., "Localization of the Exonuclease and Polymerase Domains of *Bacillus subtilis* DNA Polymerase III," Gene, 111:43-49 (1992); Barnes, et. al., "The 3'-5' Exonuclease Site of DNA Polymerase III From Gram-positive Bacteria: Definition of a Novel Motif Structure," Gene" 165:45-50 (1995) or Barnes, et al., "Purification of DNA Polymerase III of Gram-positive Bacteria," Methods in Enzy., 262:35-42 (1995)). Hence, it is possible that Pol III-L is a member of the Type I replicase (like T5) in which it is processive on its own without accessory proteins. *B. subtilis* and *S. aureus* also have a gene encoding a protein that has approximately 30% homology to the beta subunit of *E. coli*; however the protein product has not been purified or characterized (Alonso, et al., "Nucleotide Sequence of the recF Gene Cluster From *Staphylococcus aureus* and Complementation Analysis in *Bacillus subtilis* recF Mutants," Mol. Gen. Genet., 246:680-686 (1995); Alonso, et al., "Nucleotide Sequence of the recF Gene Cluster From *Staphylococcus aureus* and Complementation Analysis in *Bacillus subtilis* recF Mutants," Mol. Gen. Genet., 248:635-636 (1995)). Whether this beta subunit has a function in replication, a ring shape, or functions as a sliding clamp is not known. Even if this beta homolog is involved in replication, it is not known whether it is functional with Pol III-L or another polymerase.

There remains a need to understand the process of DNA replication in Gram positive cells at a molecular level. It is possible that a more detailed understanding of replication proteins will lead to discovery of new antibiotics. Therefore, a deeper understanding of replication proteins of Gram positive bacteria, particularly members of the *Staphylococcus* genus is especially important given the

emergence of drug resistant strains of these organisms. For example, *Staphylococcus aureus* has successfully mutated to become resistant to all common antibiotics.

The "target" protein(s) of an antibiotic drug is generally involved in a critical cell function, such that blocking its action with a drug causes the pathogenic cell to die or no longer proliferate. Current antibiotics are directed to very few targets. These include membrane synthesis proteins (e.g. vancomycin, penicillin, and its derivatives such as ampicillin, amoxicillin, and cephalosporin), the ribosome machinery (tetracycline, chloramphenicol, azithromycin, and the aminoglycosides: kanamycin, neomycin, gentamicin, streptomycin), RNA polymerase (rifampimycin), and DNA topoisomerases (novobiocin, quinolones, and fluoroquinolones). The DNA replication apparatus is a crucial life process, and, thus, the proteins involved in this process are also good targets for antibiotics.

A powerful approach to discovery of a new drug is to obtain a target protein, characterize it, and develop *in vitro* assays of its cellular function. Large chemical libraries are then screened in the functional assays to identify compounds that inhibit the target protein. These candidate pharmaceuticals are then chemically modified to optimize their potency, breadth of antibiotic spectrum, performance in animal models, non toxicity, and, finally, clinical trials. The screening of large chemical libraries requires a plentiful source of the target protein. An abundant supply of protein generally requires overproduction techniques using the gene encoding the protein. This is especially true for replication proteins as they are present in low abundance in the cell.

Selective and robust assays are needed to screen reliably a large chemical library. The assay should be insensitive to most chemicals in the concentration range normally used in the drug discovery process. These assays should also be selective and not show inhibition by antibiotics known to target proteins in processes outside of replication. The present invention is directed to overcoming these deficiencies in the art.

SUMMARY OF THE INVENTION

The present invention relates to various isolated DNA molecules from Gram positive bacteria. These include dnaE, dnaX, dnaB, PolC, dnaN, beta, and
5 dnaG DNA molecules for Gram positive bacteria. These DNA molecules can be inserted into an expression system or used to transform host cells. The isolated proteins encoded by these DNA molecules are also disclosed.

The present invention aims to understand the structure and mechanism of the chromosomal replicase of Gram positive bacteria and how it functions with a
10 helicase and primase. This knowledge and the enzymes involved in the replication process can be used for the purpose of screening for potential antibiotic drugs. Further, information about chromosomal replicases may be useful in DNA sequencing, polymerase chain reaction, and other DNA polymerase related techniques.

15 The present invention identifies the type of replicase that Gram positive bacteria employ for chromosome replication. Rather than use a DNA polymerase that attains high efficiency on its own, or with one other subunit, the Gram positive bacteria replicase is the Pol III-type of replicase (class III) that uses a sliding clamp protein. The Gram positive bacteria replicase also uses a clamp loader
20 component that assembles the sliding clamp onto DNA.

The present invention identifies two DNA polymerases (both of Pol III type) in Gram positive bacteria that utilize the sliding clamp and clamp loader. The invention also identifies a gene with homology to the alpha subunit of *E. coli* DNA polymerase III holoenzyme, the chromosomal replicase of *E. coli*. These DNA
25 polymerases can extend a primer around a large circular natural template when the beta clamp has been assembled onto the primed ssDNA by the clamp loader or a primer on a linear DNA where the beta clamp may assemble by itself by sliding over an end.

The present invention shows that the clamp and clamp loader
30 components of Gram negative cells can be exchanged for those of Gram positive cells in that the clamp, once assembled onto DNA, will function with Pol III obtained from either Gram positive and Gram negative sources. This result implies that important

contacts between the polymerase and clamp have been conserved during evolution. Therefore, these "mixed systems" may provide assays for an inhibitor of this conserved interaction. Such an inhibitor may be expected to shut down replication, and since the interaction is apparently conserved across the evolutionary spectrum
5 from Gram positive and Gram negative cells, the inhibitor may exhibit a broad spectrum of antibiotic activity. Further, these "mixed" systems are composed of all overexpressed and purified proteins (8 total; 1 from *S. aureus* and 7 from *E. coli*) making possible large quantities of protein needed for high throughput screening of hundreds of thousands of chemicals.

10 The present invention demonstrates that Gram positive bacteria contain a beta subunit that behaves as a sliding clamp that encircles DNA. A dnaX gene sequence encoding a protein homolog of the gamma/tau subunit of the clamp loader (gamma complex) *E. coli* DNA polymerase III holoenzyme is also identified. The presence of this gene confirms the presence of a clamp loading apparatus in Gram
15 positive bacteria that will assemble beta clamps onto DNA for the DNA polymerases.

A new gene sequence encoding a DNA polymerase homologous to the alpha subunit of DNA polymerase III holoenzyme of *E. coli* (referred to herein as dnaE homolog) is also identified.

Also identified is a new gene sequence encoding a homolog of the
20 replicative dnaB helicase of *E. coli*.

This application also outlines methods and assays for use of these replication proteins in drug screening processes.

BRIEF DESCRIPTION OF THE DRAWINGS

25

Figure 1 shows the construction of the *S. aureus* Pol III-L expression vector. The gene encoding Pol III-L was cloned into a pET11 expression vector in a three step cloning scheme as illustrated.

Figures 2A-C describe the expression and purification of *S. aureus* Pol
30 III-L. Figure 2A compares *E. coli* cells that contain the pET11PolC expression vector that are either induced or uninduced for protein expression. The gel is stained with Coomassie Blue. The induced band corresponds to the expected molecular weight of

the *S. aureus* Pol III-L, and is indicated to the right of the gel. Figure 2B shows the results of the MonoQ chromatography of a lysate of *E. coli* (pET11PolC-L) induced for Pol III-L. The fractions were analyzed in a Coomassie Blue stained gel (top) and for DNA synthesis (bottom). Fractions containing Pol III-L are indicated. In

5 Figure 2C, fractions containing Pol III-L from the MonoQ column were pooled and chromatographed on a phosphocellulose column. This shows an analysis of the column fractions from the phosphocellulose column in a Coomassie Blue stained polyacrylamide gel. The position of Pol III-L is indicated to the right.

Figure 3 shows the *S. aureus* beta expression vector. The *dnaN* gene
10 was amplified from *S. aureus* genomic DNA and cloned into the pET16 expression vector.

Figures 4A-C describe the expression and purification of *S. aureus* beta. Figure 4A compares *E. coli* cells that contain the pET16beta expression vector that are either induced or uninduced for protein expression. The gel is stained with
15 Coomassie Blue. The induced band corresponds to the expected molecular weight of the *S. aureus* beta, and is indicated to the right of the gel. The migration position of size standards are indicated to the left. Figure 4B shows the results of MonoQ chromatography of an *E. coli* (pET16beta) lysate induced for beta. The fractions were analyzed in a Coomassie Blue stained gel, and fractions containing beta are indicated.
20 In Figure 4C, fractions containing beta from the MonoQ column were pooled and chromatographed on a phosphocellulose column. This shows an analysis of the column fractions from the phosphocellulose column in a Coomassie Blue stained polyacrylamide gel. The position of beta is indicated to the right.

Figures 5A-B demonstrate that the *S. aureus* beta stimulates *S. aureus*
25 Pol III-L and *E. coli* Pol III core on linear DNA, but not circular DNA. In Figure 5A, the indicated proteins were added to replication reactions containing polydA-oligodT as described in the Examples *supra*. Amounts of proteins added, when present, were: lanes 1,2: *S. aureus* Pol III-L, 7.5 ng; *S. aureus* b, 6.2 ug; Lanes 3,4: *E. coli* Pol III core, 45 ng; *S. aureus* b, 9.3 ug; Lanes 5,6: *E. coli* Pol III core, 45 ng; *E. coli* b, 5µg.
30 Total DNA synthesis was: Lanes 1-6: 4.4, 30.3, 5.1, 35.5, 0.97, 28.1 pmol, respectively. In Figure 5B, Lanes 1-3, the indicated proteins were added to replication reactions containing circular singly primed M13mp18 ssDNA as described

in the Example *supra*. *S. aureus* b, 0.8 ug; *S. aureus* Pol III-L, 300 ng (purified through MonoQ); *E. coli* gamma complex, 1.7 μ g. Results in the *E. coli* system are shown in Lanes 4-6. Total DNA synthesis was: Lanes 1-6: 0.6, 0.36, 0.99, 2.7, 3.5, 280 pmol, respectively.

5 Figure 6 shows that *S. aureus* Pol III-L functions with *E. coli* beta and gamma complex on circular primed DNA. It also shows that *S. aureus* beta does not convert Pol III-L with sufficient processivity to extend the primer all the way around a circular DNA. Replication reactions were performed on the circular singly primed M13mp18 ssDNA. Proteins added to the assay were as indicated in this figure. The
10 amount of each protein, when present, was: *S. aureus* beta, 800 ng; *S. aureus* Pol III-L, 1500 ng (MonoQ fraction 64); *E. coli* Pol III core, 450 ng; *E. coli* beta, 100 ng; *E. coli* gamma complex, 1720 ng. Total DNA synthesis in each assay is indicated at the bottom of the figure.

 Figures 7A-B show that *S. aureus* contains four distinct DNA
15 polymerases. Four different DNA polymerases were partially purified from *S. aureus* cells. *S. aureus* cell lysate was separated from DNA and, then, chromatographed on a MonoQ column. Fractions were analyzed for DNA polymerase activity. Three peaks of activity were observed. The second peak was the largest and was expected to be a mixture of two DNA polymerases based on early studies in *B. subtilis*.
20 Chromatography of the second peak on phosphocellulose (Figure 7B) resolved two DNA polymerases from one another.

 Figures 8A-B show that *S. aureus* has two DNA Pol III's. The four DNA polymerases partially purified from *S. aureus* extract, designated peaks I-IV in Figure 7, were assayed on circular singly primed M13mp18 ssDNA coated with *E. coli* SSB either in the presence or absence of *E. coli* beta (50ng) and gamma complex (50 ng). Each reaction contained 2 μ l of the partially pure polymerase (Peak 1 was Mono Q fraction 24 (1.4 ug), Peak 2 was phosphocellulose fraction 26 (0.016 mg/ml), Peak 3 was phosphocellulose fraction 46 (0.18 mg/ml), and Peak 4 was MonoQ
25 fraction 50 (1 ug). Figure 8A shows the product analysis in an agarose gel.
30 Figure 8B shows the extent of DNA synthesis in each assay.

 Figure 9 compares the homology between the polypeptide encoded by dnaE of *S. aureus* and other organisms. An alignment is shown for the amino acid

sequence of the *S. aureus* dnaE product with the dnaE products (alpha subunits) of *E. coli* and *Salmonella typhimurium*.

Figure 10 compares the homology between the N-terminal regions of the gamma/tau polypeptides of *S. aureus*, *B. subtilis*, and *E. coli*. The conserved ATP site and the cystines forming the zinc finger are indicated above the sequence. The organisms used in the alignment were: *E. coli* (GenBank); and *B. subtilis*.

Figure 11 compares the homology between the DnaB polypeptide of *S. aureus* and other organisms. The organisms used in the alignment were: *E. coli* (GenBank); *B. subtilis*; *Sal. Typ.*, (*Salmonella typhimurium*).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to various isolated DNA molecules from Gram positive bacteria. These include dnaE, dnaX, dnaB, PolC, dnaN, and dnaG DNA molecules from Gram positive bacteria. These DNA molecules can be inserted into an expression system or used to transform host cells. The isolated proteins encoded by these DNA molecules are also disclosed.

These DNA molecules and proteins can be derived from any Gram positive bacteria, including *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Mycobacterium*. It is particularly directed to such DNA molecules and proteins derived from *Staphylococcus* bacteria, particularly *Staphylococcus aureus*.

One aspect of the present invention relates to a newly discovered Pol III gene of *S. aureus* cells (herein identified as dnaE) that is homologous to *E. coli* alpha (product of dnaE gene). The partial DNA sequence of the *S. aureus* dnaE gene is as follows (SEQ. ID. No. 1):

```
GATATAGATA TGGACTGGGA AGATACACGC CGAGAAAAGG TCATTCAGTA CGTCCAAGAA      60
AAATATGGCG AGCTACATGT ATCTGGAATT GTGACTTTCG GTCATCTGCT TGCAAAAGCG      120
GTTGCTAAAG ATGTTGGACG AATTATGGGG TTTGATGAAG TTACATTAAA TGAAATTTCA      180
AGTTTAATCC CACATAAATT AGGAATTACA CTTGATGAAG CATATCAAAT TGACGATTTT      240
AAAAAGTTTG TACATCGAAA CCATCGACAT CAACGCTGGT TCAGTATTTG TAAAAAGTTA      300
GAAGGTTTAC CAAGACATAC ATCTACACAT GCGGCAGGAA TTATTATTAA TGACCATCCA      360
```

	TTATATGAAT ATGCCCCTTT AACGAAAGGG GATACAGGAT TATTAACGCA ATGGACAATG	420
5	ACTGAAGCCG AACGTATTGG TTTATTAAAA ATAGATTTTC TAGGGTTACG AAATTTATCA	480
	ATTATCCATC AAATTTTGAC TCGAGTCGAA AAAGATTTAG GTTTTAATAT TGATATTGAA	540
	AAAMTTCCAT TTGATGATCA AAAAGTGTGT GAATTGTTGT CGCAAGGAGA TACGACTGGC	600
10	ATATTTCAAT TAGAGTCTGA CGGTGTTAGA AGTGTATTAA AAAAATTAAA GCCGGAACAC	660
	TTTGAGGATA TTGTTGCTGT AACTTCTTTG TATAGACCAG GTCCAATGGA AGAAATTCCA	720
15	ACTTACATTA CAAGAAGACA TGATCCAAGC AAAGTTCAAT ATTTACATCC CCATTTAGAA	780
	CCTATATTAA AAAATACTTA CGGTGTTATT ATTTATCAAG AACAAATTAT GCAAATAGCG	840
	AGCACTTTTG CAAACTTCAG TTATGGTGAA GCGGATATTT TAAGAAGAGC AATGAGTAAA	900
20	AAAAATAGAG CTGTTCTTGA AAGAGACGCT CAACATTTTA TAGAAGGTAC AAAGCAAAAT	960
	GGTTATCAGC AAGACTTAGT AAGTAAGCAG ATATTTGATT TGATTCTGAA ATTTGCTGAT	1020
25	GATGGATTTC CTAGAGCACA TGCTGTCAGC TATTCTAAAA TTGCATACAT TATGAGCTTT	1080
	TTAAAAGTCC ATTATCCAAA TTATTTTAC GCAAATATTT TAAGTAATGT TATTGGAAGT	1140
	GAGAAGAAAA CTGCTCAAAT GATAGAAGAA GCAAAAAAAC AAGGTATCAC TATATTGCCA	1200
30	CCGAACATTA ACGAAAGTCA TTGGTTTTAT AAACCTTCCC AAGAAGGCAT TTATTTATCA	1260
	ATTGGTACAA TTAAAGGTGT AGGTATCAA AGTGTGAAAG TGATTGTTGA AGAACGTTTT	1320
35	CAGAACGGCA AATTTAAAGA TTTCTTTGAT TCTGCTAGAC GTATACCGAA GAGAGTCAAA	1380
	ACGAGAAAGT TACTTGAAGC ATTGATTTTA GTGGGAGCGT TTGATGCTTT TGGTAAAACA	1440
	CGTTCAACGT TGTTCGAAGC TATTGATCAA GTGTTGGATG GTGATTTAAA CATTGAACAA	1500
40	GATGGTTTTT TATTTGATAT TTAAACGCCA AAACAGATGT ATGAAGATAA AGAAGAATTG	1560
	CCTGATGCAC TTATTAGTCA GTATGAAAAA GAATATTTAG GATTTTATGT TTCGCAACAC	1620
45	CCAGTAGATA AGAAGTTTGT TGCCAAACAA TATTTAACGA TATTTTCTTG CGAAAACGTT	1680
	GCTAAAGATG TTCGACGAAT TATGGGGTTT GATGAAGTTA AACAAA	1726

The *S. aureus* dnaE encoded protein has a partial amino acid sequence
 50 as follows (SEQ. ID. No. 2):

	Asp Ile Asp Met Asp Trp Glu Asp Thr Arg Arg Glu Lys Val Ile Gln	
	1 5 10 15	
55	Tyr Val Gln Glu Lys Tyr Gly Glu Leu His Val Ser Gly Ile Val Thr	
	20 25 30	

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	Phe Gly His Leu Leu Ala Lys Ala Val Ala Lys Asp Val Gly Arg Ile	
	35 40 45	
5	Met Gly Phe Asp Glu Val Thr Leu Asn Glu Ile Ser Ser Leu Ile Pro	
	50 55 60	
	His Lys Leu Gly Ile Thr Leu Asp Glu Ala Tyr Gln Ile Asp Asp Phe	
	65 70 75 80	
10	Lys Lys Phe Val His Arg Asn His Arg His Gln Arg Trp Phe Ser Ile	
	85 90 95	
	Cys Lys Lys Leu Glu Gly Leu Pro Arg His Thr Ser Thr His Ala Ala	
15	100 105 110	
	Gly Ile Ile Ile Asn Asp His Pro Leu Tyr Glu Tyr Ala Pro Leu Thr	
	115 120 125	
20	Lys Gly Asp Thr Gly Leu Leu Thr Gln Trp Thr Met Thr Glu Ala Glu	
	130 135 140	
	Arg Ile Gly Leu Leu Lys Ile Asp Phe Leu Gly Leu Arg Asn Leu Ser	
	145 150 155 160	
25	Ile Ile His Gln Ile Leu Thr Arg Val Glu Lys Asp Leu Gly Phe Asn	
	165 170 175	
	Ile Asp Ile Glu Lys Ile Pro Phe Asp Asp Gln Lys Val Phe Glu Leu	
30	180 185 190	
	Leu Ser Gln Gly Asp Thr Thr Gly Ile Phe Gln Leu Glu Ser Asp Gly	
	195 200 205	
35	Val Arg Ser Val Leu Lys Lys Leu Lys Pro Glu His Phe Glu Asp Ile	
	210 215 220	
	Val Ala Val Thr Ser Leu Tyr Arg Pro Gly Pro Met Glu Glu Ile Pro	
	225 230 235 240	
40	Thr Tyr Ile Thr Arg Arg His Asp Pro Ser Lys Val Gln Tyr Leu His	
	245 250 255	
	Pro His Leu Glu Pro Ile Leu Lys Asn Thr Tyr Gly Val Ile Ile Tyr	
45	260 265 270	
	Gln Glu Gln Ile Met Gln Ile Ala Ser Thr Phe Ala Asn Phe Ser Tyr	
	275 280 285	
50	Gly Glu Ala Asp Ile Leu Arg Arg Ala Met Ser Lys Lys Asn Arg Ala	
	290 295 300	
	Val Leu Glu Arg Asp Ala Gln His Phe Ile Glu Gly Thr Lys Gln Asn	
	305 310 315 320	

Gly Tyr His Glu Asp Ile Ser Lys Gln Ile Phe Asp Leu Ile Leu Lys
 325 330 335
 5 Phe Ala Asp Gly Phe Pro Arg Ala His Ala Val Ser Tyr Ser Lys Ile
 340 345 350
 Ala Tyr Ile Met Ser Phe Leu Lys Val His Tyr Pro Asn Tyr Phe Tyr
 355 360 365
 10 Ala Asn Ile Leu Ser Asn Val Ile Gly Ser Glu Lys Lys Thr Ala Gln
 370 375 380
 Met Ile Glu Glu Ala Lys Lys Gln Gly Ile Thr Ile Leu Pro Pro Asn
 385 390 395 400
 15 Ile Asn Glu Ser His Trp Phe Tyr Lys Pro Ser Gln Glu Gly Ile Tyr
 405 410 415
 Leu Ser Ile Gly Thr Ile Lys Gly Val Gly Tyr Gln Ser Val Lys Val
 420 425 430
 Ile Val Glu Glu Arg Phe Gln Asn Gly Lys Phe Lys Asp Phe Phe Asp
 435 440 445
 25 Ser Ala Arg Arg Ile Pro Lys Arg Val Lys Thr Arg Lys Leu Leu Glu
 450 455 460
 Ala Leu Ile Leu Val Gly Ala Phe Asp Ala Phe Gly Lys Thr Arg Ser
 465 470 475 480
 30 Thr Leu Leu Gln Ala Ile Asp Gln Val Leu Asp Gly Asp Leu Asn Ile
 485 490 495
 Glu Gln Asp Gly Phe Leu Phe Asp Ile Leu Thr Pro Lys Gln Met Tyr
 500 505 510
 Glu Asp Lys Glu Glu Leu Pro Asp Ala Leu Ile Ser Gln Tyr Glu Lys
 515 520 525
 40 Glu Tyr Leu Gly Phe Tyr Val Ser Gln His Pro Val Asp Lys Lys Phe
 530 535 540
 Val Ala Lys Gln Tyr Leu Thr Ile Phe Ser Cys Glu Asn Val Ala Lys
 545 550 555 560
 45 Asp Val Arg Arg Ile Met Gly Phe Asp Glu Val Lys Gln
 565 570

50 The present invention also relates to the *S. aureus* dnaX gene. This
S. aureus dnaX gene has a partial nucleotide sequence as follows (SEQ. ID. No. 3):

	TTGAATTATC AAGCCTTATA TCGTATGTAC AGACCCCAAA GTTTCGAGGA TGTGTCGGA	60
	CAAGAACATG TCACGAAGAC ATTGCGCAAT GCGATTTCGA AAGAAAAACA GTCGCATGCA	120
5	TATATTTTTA GTGGTCCGAG AGGTACGGGG AAAACGAGTA TTGCCAAAGT GTTTGCTAAA	180
	GCAATCAACT GTTTAAATAG CACTGATGGA GAACCTTGTA ATGAATGTCA TATTTGTAAA	240
10	GGCATTACGC AGGGGACTAA TTCAGATGTG ATAGAAATTG ATGCTGCTAG TAATAATGGC	300
	GTTGATGAAA TAAGAAATAT TAGAGACAAA GTTAAATATG CACCAAGTGA ATCGAAATAT	360
	AAAGTTTATA TTATAGATGA GGTGCACATG CTAACAACAG GTGCTTTTAA TGCCCTTTTA	420
15	AAGACGTTAG AAGAACCTCC AGCACACGCT ATTTTATAT TGGCAACGAC AGAACCACAT	480
	AAAATCCCTC CAACAATCAT TTCTAGGGCA CAACGTTTTG ATTTTAAAGC AATTAGCCTA	540
	GATCAAATTG TTGAACGTTT AAAATTTGTA GCAGATGCAC AACAAATTGA ATGTGAAGAT	600
20	GAAGCCTTGG CATTTATCGC TAAAGCGTCT GAAGGGGGTA TGCCTGATGC ATTAAGTATT	660
	ATGGATCAGG CTATTGCTTT CGGCGATGGC ACATTGACAT TACAAGATGC CCTAAATGTT	720
25	ACGGGTAGCG TTCATGATGA AGCGTTGGAT CACTTGTTTG ATGATATTGT ACAAGGTGAC	780
	GTACAAGCAT CTTTTAAAA ATACCATCAG TTTATAACAG AAGGTAAAGA AGTGAATCGC	840
30	CTAATAAATG ATATGATTTA TTTTGTCAGA GATACGATTA TGAATAAAC ATCTGAGAAA	900
	GATACTGAGT ATCGAGCACT GATGAACCTA GAATTAGATA TGTTATATCA AATGATTGAT	960
	CTTATTAATG ATACATTAGT GTCGATTGCT TTTAGTGTGA ATCAAACGT TCATTTTGAA	1020
35	GTATTGTTAG TAAAATTAGC TGAGCAGATT AAGGGTCAAC CACAAGTGAT TGCGAATGTA	1080
	GCTGAACCAG CACAAATTGC TTCATCGCCA AACACAGATG TATTGTTGCA ACGTATGGAA	1140
40	CAGTTAGAGC AAGAACTAAA AACACTAAAA GCACAAGGAG TGAGTGTTGC TCCTACTCAA	1200
	AAATCTTCGA AAAAGCCTGC GAGAGGTATA CAAAAATCTA AAAATGCATT TTCAATGCAA	1260
	CAAATTGCAA AAGTGCTAGA TAAAGCGAAT AAGGCAGATA TCAAATTGTT GAAAGATCAT	1320
45	TGGCAAGAAG TGATTGACCA TGCCCAAAAC AATGATAAAA AATCACTCGT TAGTTTATTG	1380
	CAAAATTCGG AACCTGTGGC GGCAAGTGAA GATCACGTCC TTGTGAAATT TGAGGAAGAG	1440
50	ATCCATTGTG AAATCGTCAA TAAAGACGAC GAGAAACGTA GTAGTATAGA AAGTGTTGTA	1500
	TGTAATATCG TTAATAAAAA CGTTAAAGTT GTTGGTGTAC CATCAGATCA ATGGCAAAGA	1560
	GTTCGAACGG AGTATTTACA AAATCGTAAA AACGAAGGCG ATGATATGCC AAAGCAACAA	1620
55	GCACAACAAA CAGATATTGC TCAAAAAGCA AAAGATCTTT TCGGTGAAGA AACTGTACAT	1680
	GTGATAGATG AAGAGTGA	1698

The *S. aureus* dnaX protein (i.e. the gamma subunit/tau subunit) has a partial amino acid sequence as follows (SEQ. ID. No. 4):

5	Leu Asn Tyr Gln Ala Leu Tyr Arg Met Tyr Arg Pro Gln Ser Phe Glu	1 5 10 15
	Asp Val Val Gly Gln Glu His Val Thr Lys Thr Leu Arg Asn Ala Ile	20 25 30
10	Ser Lys Glu Lys Gln Ser His Ala Tyr Ile Phe Ser Gly Pro Arg Gly	35 40 45
	Thr Gly Lys Thr Ser Ile Ala Lys Val Phe Ala Lys Ala Ile Asn Cys	50 55 60
15	Leu Asn Ser Thr Asp Gly Glu Pro Cys Asn Glu Cys His Ile Cys Lys	65 70 75 80
20	Gly Ile Thr Gln Gly Thr Asn Ser Asp Val Ile Glu Ile Asp Ala Ala	85 90 95
	Ser Asn Asn Gly Val Asp Glu Ile Arg Asn Ile Arg Asp Lys Val Lys	100 105 110
25	Tyr Ala Pro Ser Glu Ser Lys Tyr Lys Val Tyr Ile Ile Asp Glu Val	115 120 125
	His Met Leu Thr Thr Gly Ala Phe Asn Ala Leu Leu Lys Thr Leu Glu	130 135 140
30	Glu Pro Pro Ala His Ala Ile Phe Ile Leu Ala Thr Thr Glu Pro His	145 150 155 160
35	Lys Ile Pro Pro Thr Ile Ile Ser Arg Ala Gln Arg Phe Asp Phe Lys	165 170 175
	Ala Ile Ser Leu Asp Gln Ile Val Glu Arg Leu Lys Phe Val Ala Asp	180 185 190
40	Ala Gln Gln Ile Glu Cys Glu Asp Glu Ala Leu Ala Phe Ile Ala Lys	195 200 205
	Ala Ser Glu Gly Gly Met Arg Asp Ala Leu Ser Ile Met Asp Gln Ala	210 215 220
45	Ile Ala Phe Gly Asp Gly Thr Leu Thr Leu Gln Asp Ala Leu Asn Val	225 230 235 240
50	Thr Gly Ser Val His Asp Glu Ala Leu Asp His Leu Phe Asp Asp Ile	245 250 255

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	Val	Gln	Gly	Asp	Val	Gln	Ala	Ser	Phe	Lys	Lys	Tyr	His	Gln	Phe	Ile	
				260					265					270			
5	Thr	Glu	Gly	Lys	Glu	Val	Asn	Arg	Leu	Ile	Asn	Asp	Met	Ile	Tyr	Phe	
			275					280					285				
	Val	Arg	Asp	Thr	Ile	Met	Asn	Lys	Thr	Ser	Glu	Lys	Asp	Thr	Glu	Tyr	
		290					295					300					
10	Arg	Ala	Leu	Met	Asn	Leu	Glu	Leu	Asp	Met	Leu	Tyr	Gln	Met	Ile	Asp	
	305					310					315					320	
	Leu	Ile	Asn	Asp	Thr	Leu	Val	Ser	Ile	Arg	Phe	Ser	Val	Asn	Gln	Asn	
15					325					330					335		
	Val	His	Phe	Glu	Val	Leu	Leu	Val	Lys	Leu	Ala	Glu	Gln	Ile	Lys	Gly	
				340					345					350			
20	Gln	Pro	Gln	Val	Ile	Ala	Asn	Val	Ala	Glu	Pro	Ala	Gln	Ile	Ala	Ser	
			355					360						365			
	Ser	Pro	Asn	Thr	Asp	Val	Leu	Leu	Gln	Arg	Met	Glu	Gln	Leu	Glu	Gln	
		370					375					380					
25	Glu	Leu	Lys	Thr	Leu	Lys	Ala	Gln	Gly	Val	Ser	Val	Ala	Pro	Thr	Gln	
	385					390					395					400	
	Lys	Ser	Ser	Lys	Lys	Pro	Ala	Arg	Gly	Ile	Gln	Lys	Ser	Lys	Asn	Ala	
30					405					410					415		
	Phe	Ser	Met	Gln	Gln	Ile	Ala	Lys	Val	Leu	Asp	Lys	Ala	Asn	Lys	Ala	
				420					425					430			
35	Asp	Ile	Lys	Leu	Leu	Lys	Asp	His	Trp	Gln	Glu	Val	Ile	Asp	His	Ala	
		435					440						445				
	Gln	Asn	Asn	Asp	Lys	Lys	Ser	Leu	Val	Ser	Leu	Leu	Gln	Asn	Ser	Glu	
		450					455					460					
40	Pro	Val	Ala	Ala	Ser	Glu	Asp	His	Val	Leu	Val	Lys	Phe	Glu	Glu	Glu	
	465					470					475					480	
	Ile	His	Cys	Glu	Ile	Val	Asn	Lys	Asp	Asp	Glu	Lys	Arg	Ser	Ser	Ile	
45					485					490					495		
	Glu	Ser	Val	Val	Cys	Asn	Ile	Val	Asn	Lys	Asn	Val	Lys	Val	Val	Gly	
				500					505					510			
50	Val	Pro	Ser	Asp	Gln	Trp	Gln	Arg	Val	Arg	Thr	Glu	Tyr	Leu	Gln	Asn	
		515						520					525				
	Arg	Lys	Asn	Glu	Gly	Asp	Asp	Met	Pro	Lys	Gln	Gln	Ala	Gln	Gln	Thr	
		530					535					540					

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Asp Ile Ala Gln Lys Ala Lys Asp Leu Phe Gly Glu Glu Thr Val His
545 550 555 560

5 Val Ile Asp Glu Glu Gln
565

This invention also relates to the partial nucleotide sequence of the
S. aureus dnaB gene as follows (SEQ. ID. No. 5):

10
ATGGATAGAA TGTATGAGCA AAATCAAATG CCGCATAACA ATGAAGCTGA ACAGTCTGTC 60
TTAGGTTCAA TTATTATAGA TCCAGAATTG ATTAATACTA CTCAGGAAGT TTTGCTTCCT 120
15 GAGTCGTTTT ATAGGGGTGC CCATCAACAT ATTTTCCGTG CAATGATGCA CTAAATGAA 180
GATAATAAAG AAATTGATGT TGTAACATTG ATGGATCAAT TATCGACGGA AGGTACGTTG 240
20 AATGAAGCGG GTGGCCCGCA ATATCTTGCA GAGTTATCTA CAAATGTACC AACGACGCGA 300
AATGTTTCAGT ATTATACTGA TATCGTTTCT AAGCATGCAT TAAAACGTAG ATTGATTCAA 360
ACTGCAGATA GTATTGCCAA TGATGGATAT AATGATGAAC TTGAACTAGA TGCGATTTTA 420
25 AGTGATGCAG AACGTCGAAT TTTAGAGCTA TCATCTTCTC GTGAAAGCGA TGGCTTTAAA 480
GACATTCGAG ACGTCTTAGG ACAAGTGAT GAAACAGCTG AAGAGCTTGA TCAAATAGT 540
GGTCAAACAC CAGGTATACC TACAGGATAT CGAGATTTAG ACCAAATGAC AGCAGGGTTC 600
30 AACCGAAATG ATTTAATTAT CCTTGCAGCG CGTCCATCTG TAGGTAAGAC TGC GTTTCGCA 660
CTTAATATTG CACAAAAAGT TGCAACGCAT GAAGATATGT ATACAGTTAA AAGCAACAGG 720
35 AAGTTTCTGA AATCTCTCGT ACATTAAAAG CATTAGCCCG TGAATTAAAA TGTCCAGTTA 780
TCGCATTAAG TCAGTTATCT CGTGGTGTG AACAAACGACA AGATAAACGT CCAATGATGA 840
GTGATATTCG TGAATCTGGT TCGATTGAGC AAGATGCCGA TATCGTTGCA TTCTTATACC 900
40 GTGATGATTA CTATAACCGT GCGGCGGATG AAGATGATGA CGATGATGGT GGTTCGAGC 960
CACAAACGAA TGATGAAAAC GGTGAAATTG AAATTATCAT TGTTAAGCAA CGTAACGGTC 1020
45 CAACAGGCAC AGTTAAGTTA CATTTTATGA AACAAATATAA TAAATTTTAG AGCTATCATC 1080
TTTTCGTGAA AGCGATGGCT TTAAAGACAT TCGAGACGTC TTAGGACAAG TGTATGAAAC 1140
AGCTGAAGAG CTTGATCAAA ATAGTGGTCA AACACCAGGT ATACCTACAG GATATCGAGA 1200
50 TTTAGACCAA ATGACAGCAG GGTTCACCG AAATGATTTA ATTATCCTTG CAGCGCGTCC 1260
ATCTGTAGGT AAGACTGCGT TCGCACTTAA TATTGCACAA AAAGTTGCAA CGCATCCGCA 1320
55 CTTAATATTG CCAATAAGTT GGAACGCATG AAGATATATC TAGCAGTTGG TATTTTCTCA 1380
CTAGAGATGG GTGCTGATCA GTTAACCACA CGTATGATTT GTAGTTCTGG TAATGTTGAC 1440

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TCAAACCGCT TAAGAACCGG TACTATGACT GAGGAAGATT GGAGTCGTTT TACTATAGCG 1500
5 GTTGGTAAAT TATCACGTAC GAAGATTTTT ATTGATGATA CACCGGGTAT TCGAATTAAT 1560
GATTTACGTT CTAAATGTCG TCGATTAAAG CAAGAACATG GCTTAGACAT GATTGTGATT 1620
GACTACTTAC AGTTGATTCA AGGTAGTGGT TCACGTGCGT CCGATAACAG ACAACAGGAA 1680
10 GTTCTGAAA TCTCTCGTAC ATTAAAAGCA TTAGCCCGTG AATTAAAATG TCCAGTTATC 1740
GCATTAAGTC AGTTATCTCG TGGTGTGAA CAACGACAAG ATAAACGTCC AATGATGAGT 1800
GATATTCGTG AATCTGGTTC GATTGAGCAA GATGCCGATA TCGTTGCATT CTTATACCGT 1860
15 GATGATTACT ATAACCGTGG CGGCGATGAA GATGATGACG ATGATGGTGG TTTCGAGCCC 1920
CAAACGAATG ATGAAAACGG TGAAATTGAA ATTATCATTG CTAAGCAACG TTACGGTCCA 1980
20 ACAGGCACAG TTAAGTTACT TTTTATGAAA CAATATGGTA AATTTACCGA TATC 2034

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The amino acid sequence of *S. aureus* DnaB encoded by the dnaB gene is as follows (SEQ. ID. No. 6):

```

25 Met Asp Arg Met Tyr Glu Gln Asn Gln Met Pro His Asn Asn Glu Ala
   1           5           10           15
30 Glu Gln Ser Val Leu Gly Ser Ile Ile Ile Asp Pro Glu Leu Ile Asn
   20           25           30
   Thr Thr Gln Glu Val Leu Leu Pro Glu Ser Phe Tyr Arg Gly Ala His
   35           40           45
35 Gln His Ile Phe Arg Ala Met Met His Leu Asn Glu Asp Asn Lys Glu
   50           55           60
   Ile Asp Val Val Thr Leu Met Asp Gln Leu Ser Thr Glu Gly Thr Leu
   65           70           75           80
40 Asn Glu Ala Gly Gly Pro Gln Tyr Leu Ala Glu Leu Ser Thr Asn Val
   85           90           95
   Pro Thr Thr Arg Asn Val Gln Tyr Tyr Thr Asp Ile Val Ser Lys His
   100          105          110
45 Ala Leu Lys Arg Arg Leu Ile Gln Thr Ala Asp Ser Ile Ala Asn Asp
   115          120          125
50 Gly Tyr Asn Asp Glu Leu Glu Leu Asp Ala Ile Leu Ser Asp Ala Glu
   130          135          140
   Arg Arg Ile Leu Glu Leu Ser Ser Ser Arg Glu Ser Asp Gly Phe Lys
   145          150          155          160
55

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	Asp Ile Arg Asp Val Leu Gly Gln Val Tyr Glu Thr Ala Glu Glu Leu	
	165	170 175
5	Asp Gln Asn Ser Gly Gln Thr Pro Gly Ile Pro Thr Gly Tyr Arg Asp	
	180	185 190
	Leu Asp Gln Met Thr Ala Gly Phe Asn Arg Asn Asp Leu Ile Ile Leu	
	195	200 205
10	Ala Ala Arg Pro Ser Val Gly Lys Thr Ala Phe Ala Leu Asn Ile Ala	
	210	215 220
	Gln Lys Leu Glu Arg Met Lys Ile Tyr Leu Ala Val Gly Ile Phe Ser	
15	225	230 235 240
	Leu Glu Met Gly Ala Asp Gln Leu Thr Thr Arg Met Ile Cys Ser Ser	
	245	250 255
20	Gly Asn Val Asp Ser Asn Arg Leu Arg Thr Gly Thr Met Thr Glu Glu	
	260	265 270
	Asp Trp Ser Arg Phe Thr Ile Ala Val Gly Lys Leu Ser Arg Thr Lys	
	275	280 285
25	Ile Phe Ile Asp Asp Thr Pro Gly Ile Arg Ile Asn Asp Leu Arg Ser	
	290	295 300
	Lys Cys Arg Arg Leu Lys Gln Glu His Gly Leu Asp Met Ile Val Ile	
30	305	310 315 320
	Asp Tyr Leu Gln Leu Ile Gln Gly Ser Gly Ser Arg Ala Ser Asp Asn	
	325	330 335
35	Arg Gln Gln Glu Val Ser Glu Ile Ser Arg Thr Leu Lys Ala Leu Ala	
	340	345 350
	Arg Glu Leu Lys Cys Pro Val Ile Ala Leu Ser Gln Leu Ser Arg Gly	
	355	360 365
40	Val Glu Gln Arg Gln Asp Lys Arg Pro Met Met Ser Asp Ile Arg Glu	
	370	375 380
	Ser Gly Ser Ile Glu Gln Asp Ala Asp Ile Val Ala Phe Leu Tyr Arg	
45	385	390 395 400
	Asp Asp Tyr Tyr Asn Arg Gly Gly Asp Glu Asp Asp Asp Asp Gly	
	405	410 415
50	Gly Phe Glu Pro Gln Thr Asn Asp Glu Asn Gly Glu Ile Glu Ile Ile	
	420	425 430
	Ile Ala Lys Gln Arg Tyr Gly Pro Gly Thr Val Lys Leu Leu Phe Met	
	435	440 445

Lys Gln Tyr Gly Lys Phe Thr Asp Ile
450 455

The present invention also uses the gene sequence of *S. aureus* PolC (encoding Pol III-L). The nucleotide sequence is as follows (SEQ. ID. No. 7):

```

5  ATGACAGAGC AACAAAAATT TAAAGTGCTT GCTGATCAAA TTAAATTTTC AAATCAATTA      60
   GATGCTGAAA TTTTAAATTC AGGTGAACTG ACACGTATAG ATGTTTCTAA CAAAAACAGA      120
10 ACATGGGAAT TTCATATTAC ATTACCACAA TTCTTAGCTC ATGAAGATTA TTTATTATTT      180
   ATAAATGCAA TAGAGCAAGA GTTTAAAGAT ATCGCCAACG TTACATGTCG TTTTACGGTA      240
   ACAAATGGCA CGAATCAAGA TGAACATGCA ATTAAATACT TTGGGCACTG TATTGACCAA      300
15 ACAGCTTTAT CTCCAAAAGT TAAAGGTCAA TTGAAACAGA AAAAGCTTAT TATGTCTGGA      360
   AAAGTATTAA AAGTAATGGT ATCAAATGAC ATTGAACGTA ATCATTTTGA TAAGGCATGT      420
20 AATGGAAGTC TTATCAAAGC GTTTAGAAAT TGTGGTTTTG ATATCGATAA AATCATATTC      480
   GAAACAAATG ATAATGATCA AGAACAAAAC TTAGCTTCTT TAGAAGCACA TATTCAAGAA      540
   GAAGACGAAC AAAGTGCACG ATTGGCAACA GAGAACTTG AAAAAATGAA AGCTGAAAAA      600
25 GCGAACAAC AAGATAACAA GCAAAGTGCT GTCGATAAGT GTCAAATTGG TAAGCCGATT      660
   CAAATTGAAA ATATTAAACC AATTGAATCT ATTATTGAGG AAGAGTTTAA AGTTGCAATA      720
30 GAGGGTGTCA TTTTGTATAT AAACCTAAAA GAACCTAAAA GTGGTCGCCA TATCGTAGAA      780
   ATTAAAGTGA CTGACTATAC GGA CTCTTTA GTTTTAAAAA TGTTTACTCG TAAAAACAAA      840
   GATGATTTAG AACATTTTAA AGCGCTAAGT GTTGGTAAAT GGGTTAGGGC TCAAGGTCGT      900
35 ATTGAAGAAG ATACATTTAT TAGAGATTTA GTTATGATGA TGTCTGATAT TGAAGAGATT      960
   AAAAAAGCGA CAAAAAAGA TAAGGCTGAA GAAAAGCGAG TAGAATTCCA CTTGCATACT      1020
40 GCAATGAGCC AAATGGATGG TATACCCAAT ATTGGTGCCT ATGTTAAACA GGCAGCAGAC      1080
   TGGGGACATC CAGCCATTGC GGTACAGAC CATAATGTGG TGCAAGCATT TCCAGATGCT      1140
   CACGCAGCAG CGGAAAAACA TGGCATTAAA ATGATATACG GTATGGAAGG TATGTTAGTT      1200
45 GATGATGGTG TTCCGATTGC ATACAAACCA CAAGATGTCG TATTAAAAGA TGCTACTTAT      1260
   GTTGTGTTTC ACGTTGAGAC AACTGGTTTA TCAAATCAGT ATGATAAAAT CATCGAGCTT      1320
50 GCAGCTGTGA AAGTTCATAA CGGTGAAATC ATCGATAAGT TTGAAAGGTT TAGTAATCCG      1380
   CATGAACGAT TATCGGAAAC GATTATCAAT TTGACGCATA TTACTGATGA TATGTTAGTA      1440
   GATGCCCCTG AGATTGAAGA AGTACTTACA GAGTTTAAAG AATGGGTTGG CGATGCGATA      1500
55 TTCGTAGCGC ATAATGCTTC GTTTGATATG GGCTTCATCG ATACGGGATA TGAACGTCTT      1560

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	GGGTTTGAC CATCAACGAA TGSTGTTATC GATACTTTAG AATTATCTCG TACGATTAAT	1620
	ACTGAATATG GTAAACATGG TTTGAATTTC TTGGCTAAAA AATATGGCGT AGAATTAACG	1680
5	CAACATCACC GTGCCATTTA TGATACAGAA GCAACAGCTT ACATTTTCAT AAAAATGGTT	1740
	CAACAAATGA AAGAATTAGG CGTATTAAAT CATAACGAAA TCAACAAAAA ACTCAGTAAT	1800
10	GAAGATGCAT ATAAACGTGC AAGACCTAGT CATGTCACAT TAATTGTACA AAACCAACAA	1860
	GGTCTTAAAA ATCTATTTAA AATTGTAAGT GCATCATTGG TGAAGTATTT CTACCGTACA	1920
	CCTCGAATTC CACGTCATT GTTAGATGAA TATCGTGAGG GATTATTGGT AGGTACAGCG	1980
15	TGTGATGAAG GTGAATTATT TACGGCAGTT ATGCAGAAGG ACCAGAGTCA AGTTGAAAAA	2040
	ATTGCCAAAT ATTATGATTT TATTGAAATT CAACCCCGG CACTTTATCA AGATTTAATT	2100
20	GATAGAGAGC TTATTAGAGA TACTGAAACA TTACATGAAA TTTATCAACG TTTAATACAT	2160
	GCAGGTGACA CAGCGGGTAT ACCTGTTATT GCGACAGGAA ATGCACACTA TTTGTTTGAA	2220
	CATGATGGTA TCGCACGTAA AATTTTAATA GCATCACAAC CCGGCAATCC ACTTAATCGC	2280
25	TCAACTTTAC CGGAAGCACA TTTTAGAACT ACAGATGAAA TGTAAACGA GTTTCATTTT	2340
	TTAGGTGAAG AAAAAGCGCA TGAAATTGTT GTGAAAAATA CAAACGAATT AGCAGATCGA	2400
30	ATTGAACGTG TTGTTCTTAT TAAAGATGAA TTATACACAC CGCGTATGGA AGGTGCTAAC	2460
	GAAGAAATTA GAGAACTAAG TTATGCAAAT GCGCGTAAAC TGTATGGTGA AGACCTGCCT	2520
	CAAATCGTAA TTGATCGATT AGAAAAAGAA TTAAAAAGTA TTATCGGTAA TGGATTTGCG	2580
35	GTAATTTACT TAATTTTCGCA ACGTTTAGTT AAAAAATCAT TAGATGATGG ATACTTAGTT	2640
	GGTTCCCGTG GTTCAGTAGG TTCTAGTTTT GTAGCGACAA TGACTGAGAT TACTGAAGTA	2700
40	AACCCGTTAC CGCCACACTA TATTTGTCCG AACTGTAAAA CGAGTGAATT TTCAATGAT	2760
	GGTTCAGTAG GATCAGGATT TGATTTACCT GATAAGACGT GTGAACTTG TGGAGCGCCA	2820
	CTTATTAAAG AAGGACAAGA TATTCGGTTT GAAAAATTTT TAGGATTTAA GGGAGATAAA	2880
45	GTTCTTGATA TCGACTTAAA CTTTAGTGGT GAATATCAAC CGAATGCCCC TAACTACACA	2940
	AAAGTATTAT TTGGTGAGGA TAAAGTATTC CGTGCAGGTA CAATTGGTAC TGTGCTGAA	3000
50	AAGACTGCTT TTGTTATGT TAAAGGTTAT TTGAATGATC AAGGTATCCA CAAAAGAGGT	3060
	GCTGAAATAG ATCGACTCGT TAAAGGATGT ACAGGTGTAC CTGATTACAT GGATATTTAT	3120
	GATTTTACGC CGATACAATA TCCTGCCGAT GATCAAAATT CAGCATGGAT GACGACACAT	3180
55	TTTGATTTCC ATTCTATTCA TGATAATGTA TTAAACTTG ATATACTTGG ACACGATGAT	3240
	CCAACAATGA TTCGTATGCT TCAAGATTTA TCAGGAATTG ATCCAAAAAC AATACCTGTA	3300
60	GATGATAAAG AAGTTATGCA GATATTTAGT ACACCTGAAA GTTTGGGTGT TACTGAAGAT	3360
	GAAATTTTAT GTAAAACAGG TACATTTGGG GTACCGAATT CGGACAGGAT TCGTCGTCAA	3420

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ATGTTAGAAG ATACAAAGCC AACAACATTT TCTGAATTAG TTCAAATCTC AGGATTATCT 3480
5 CATGGTACAG ATGTGTGGTT AGGCAATGCT CAAGAATTAA TTAAAACCGG TATATGTGAT 3540
TTATCAAGTG TAATTGGTTG TCGTGATGAT ATCATGGTTT ATTTAATGTA TGCTGGTTTA 3600
GAACCATCAA TGGCTTTTAA AATAATGGAG TCAGTACGTA AAGGTAAAGG TTAACTGAA 3660
10 GAAATGATTG AAACGATGAA AGAAAATGAA GTGCCAGATT GGTATTTAGA TTCATGTCTT 3720
AAAATTAAGT ACATATTCCC TAAAGCCCAT GCAGCAGCAT ACGTTTAAAT GGCAGTACGT 3780
ATCGCATATT TCAAAGTACA TCATCCACTT TATTACTATG CATCTTACTT TACAATTCGT 3840
15 GCGTCAGACT TTGATTTAAT CACGATGATT AAAGATAAAA CAAGCATTCG AAATACTGTA 3900
AAAGACATGT ATTCTCGCTA TATGGATCTA GGTAAGGACG ATTAACAGTC 3960
20 TTGGAAATTA TGAATGAAAT GGCGCATCGA GGTATTCGAA TGCAACCGAT TAGTTTAGAA 4020
AAGAGTCAGG CGTTCGAATT TATCATTGAA GGCGATACAC TTATCCGCC GTTCATATCA 4080
GTGCCTGGGC TTGGCGAAAA CGTTGCGAAA CGAATTGTTG AAGCTCGTGA CGATGGCCCA 4140
25 TTTTATCAA AAGAAGATTT AAACAAAAAA GCTGGATTAT ATCAGAAAAT TATTGAGTAT 4200
TTAGATGAGT TAGGCTCATT ACCGAATTTA CCAGATAAAG CTCAACTTTC GATATTTGAT 4260
30 ATGTAA 4266

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The amino acid sequence of the *S. aureus* PolC gene product, Pol III-L is as follows (SEQ. ID. No. 8):

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35 Met Thr Glu Gln Gln Lys Phe Lys Val Leu Ala Asp Gln Ile Lys Ile
   1           5           10           15
40 Ser Asn Gln Leu Asp Ala Glu Ile Leu Asn Ser Gly Glu Leu Thr Arg
   20           25           30
45 Ile Asp Val Ser Asn Lys Asn Arg Thr Trp Glu Phe His Ile Thr Leu
   35           40           45
50 Pro Gln Phe Leu Ala His Glu Asp Tyr Leu Leu Phe Ile Asn Ala Ile
   50           55           60
   Glu Gln Glu Phe Lys Asp Ile Ala Asn Val Thr Cys Arg Phe Thr Val
   65           70           75           80
55 Thr Asn Gly Thr Asn Gln Asp Glu His Ala Ile Lys Tyr Phe Gly His
   85           90           95
   Cys Ile Asp Gln Thr Ala Leu Ser Pro Lys Val Lys Gly Gln Leu Lys
   100          105          110
60 Gln Lys Lys Leu Ile Met Ser Gly Lys Val Leu Lys Val Met Val Ser
   115          120          125

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	Asn Asp Ile Glu Arg Asn His Phe Asp Lys Ala Cys Asn Gly Ser Leu	
	130	135 140
5	Ile Lys Ala Phe Arg Asn Cys Gly Phe Asp Ile Asp Lys Ile Ile Phe	
	145	150 155 160
	Glu Thr Asn Asp Asn Asp Gln Glu Gln Asn Leu Ala Ser Leu Glu Ala	
10		165 170 175
	His Ile Gln Glu Glu Asp Glu Gln Ser Ala Arg Leu Ala Thr Glu Lys	
		180 185 190
	Leu Glu Lys Met Lys Ala Glu Lys Ala Lys Gln Gln Asp Asn Lys Gln	
15		195 200 205
	Ser Ala Val Asp Lys Cys Gln Ile Gly Lys Pro Ile Gln Ile Glu Asn	
		210 215 220
20	Ile Lys Pro Ile Glu Ser Ile Ile Glu Glu Glu Phe Lys Val Ala Ile	
		225 230 235 240
	Glu Gly Val Ile Phe Asp Ile Asn Leu Lys Glu Leu Lys Ser Gly Arg	
25		245 250 255
	His Ile Val Glu Ile Lys Val Thr Asp Tyr Thr Asp Ser Leu Val Leu	
		260 265 270
	Lys Met Phe Thr Arg Lys Asn Lys Asp Asp Leu Glu His Phe Lys Ala	
30		275 280 285
	Leu Ser Val Gly Lys Trp Val Arg Ala Gln Gly Arg Ile Glu Glu Asp	
		290 295 300
35	Thr Phe Ile Arg Asp Leu Val Met Met Met Ser Asp Ile Glu Glu Ile	
		305 310 315 320
	Lys Lys Ala Thr Lys Lys Asp Lys Ala Glu Glu Lys Arg Val Glu Phe	
40		325 330 335
	His Leu His Thr Ala Met Ser Gln Met Asp Gly Ile Pro Asn Ile Gly	
		340 345 350
45	Ala Tyr Val Lys Gln Ala Ala Asp Trp Gly His Pro Ala Ile Ala Val	
		355 360 365
	Thr Asp His Asn Val Val Gln Ala Phe Pro Asp Ala His Ala Ala Ala	
		370 375 380
50	Glu Lys His Gly Ile Lys Met Ile Tyr Gly Met Glu Gly Met Leu Val	
		385 390 395 400
	Asp Asp Gly Val Pro Ile Ala Tyr Lys Pro Gln Asp Val Val Leu Lys	
55		405 410 415
	Asp Ala Thr Tyr Val Val Phe Asp Val Glu Thr Thr Gly Leu Ser Asn	
		420 425 430
60	Gln Tyr Asp Lys Ile Ile Glu Leu Ala Ala Val Lys Val His Asn Gly	
		435 440 445

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	Glu Ile Ile Asp Lys Phe Glu Arg Phe Ser Asn Pro His Glu Arg Leu	
	450	455 460
5	Ser Glu Thr Ile Ile Asn Leu Thr His Ile Thr Asp Asp Met Leu Val	
	465	470 475 480
	Asp Ala Pro Glu Ile Glu Glu Val Leu Thr Glu Phe Lys Glu Trp Val	
		485 490 495
10	Gly Asp Ala Ile Phe Val Ala His Asn Ala Ser Phe Asp Met Gly Phe	
		500 505 510
	Ile Asp Thr Gly Tyr Glu Arg Leu Gly Phe Gly Pro Ser Thr Asn Gly	
15		515 520 525
	Val Ile Asp Thr Leu Glu Leu Ser Arg Thr Ile Asn Thr Glu Tyr Gly	
		530 535 540
20	Lys His Gly Leu Asn Phe Leu Ala Lys Lys Tyr Gly Val Glu Leu Thr	
		545 550 555 560
	Gln His His Arg Ala Ile Tyr Asp Thr Glu Ala Thr Ala Tyr Ile Phe	
		565 570 575
25	Ile Lys Met Val Gln Gln Met Lys Glu Leu Gly Val Leu Asn His Asn	
		580 585 590
	Glu Ile Asn Lys Lys Leu Ser Asn Glu Asp Ala Tyr Lys Arg Ala Arg	
30		595 600 605
	Pro Ser His Val Thr Leu Ile Val Gln Asn Gln Gln Gly Leu Lys Asn	
		610 615 620
35	Leu Phe Lys Ile Val Ser Ala Ser Leu Val Lys Tyr Phe Tyr Arg Thr	
		625 630 635 640
	Pro Arg Ile Pro Arg Ser Leu Leu Asp Glu Tyr Arg Glu Gly Leu Leu	
		645 650 655
40	Val Gly Thr Ala Cys Asp Glu Gly Glu Leu Phe Thr Ala Val Met Gln	
		660 665 670
	Lys Asp Gln Ser Gln Val Glu Lys Ile Ala Lys Tyr Tyr Asp Phe Ile	
45		675 680 685
	Glu Ile Gln Pro Pro Ala Leu Tyr Gln Asp Leu Ile Asp Arg Glu Leu	
		690 695 700
50	Ile Arg Asp Thr Glu Thr Leu His Glu Ile Tyr Gln Arg Leu Ile His	
		705 710 715 720
	Ala Gly Asp Thr Ala Gly Ile Pro Val Ile Ala Thr Gly Asn Ala His	
		725 730 735
55	Tyr Leu Phe Glu His Asp Gly Ile Ala Arg Lys Ile Leu Ile Ala Ser	
		740 745 750
	Gln Pro Gly Asn Pro Leu Asn Arg Ser Thr Leu Pro Glu Ala His Phe	
60		755 760 765

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	Arg Thr Thr Asp Glu Met Leu Asn Glu Phe His Phe Leu Gly Glu Glu	
	770	775 780
5	Lys Ala His Glu Ile Val Val Lys Asn Thr Asn Glu Leu Ala Asp Arg	
	785	790 795 800
	Ile Glu Arg Val Val Pro Ile Lys Asp Glu Leu Tyr Thr Pro Arg Met	
		805 810 815
10	Glu Gly Ala Asn Glu Glu Ile Arg Glu Leu Ser Tyr Ala Asn Ala Arg	
		820 825 830
	Lys Leu Tyr Gly Glu Asp Leu Pro Gln Ile Val Ile Asp Arg Leu Glu	
15		835 840 845
	Lys Glu Leu Lys Ser Ile Ile Gly Asn Gly Phe Ala Val Ile Tyr Leu	
		850 855 860
20	Ile Ser Gln Arg Leu Val Lys Lys Ser Leu Asp Asp Gly Tyr Leu Val	
		865 870 875 880
	Gly Ser Arg Gly Ser Val Gly Ser Ser Phe Val Ala Thr Met Thr Glu	
		885 890 895
25	Ile Thr Glu Val Asn Pro Leu Pro Pro His Tyr Ile Cys Pro Asn Cys	
		900 905 910
	Lys Thr Ser Glu Phe Phe Asn Asp Gly Ser Val Gly Ser Gly Phe Asp	
30		915 920 925
	Leu Pro Asp Lys Thr Cys Glu Thr Cys Gly Ala Pro Leu Ile Lys Glu	
		930 935 940
35	Gly Gln Asp Ile Pro Phe Glu Lys Phe Leu Gly Phe Lys Gly Asp Lys	
		945 950 955 960
	Val Pro Asp Ile Asp Leu Asn Phe Ser Gly Glu Tyr Gln Pro Asn Ala	
		965 970 975
40	His Asn Tyr Thr Lys Val Leu Phe Gly Glu Asp Lys Val Phe Arg Ala	
		980 985 990
	Gly Thr Ile Gly Thr Val Ala Glu Lys Thr Ala Phe Gly Tyr Val Lys	
45		995 1000 1005
	Gly Tyr Leu Asn Asp Gln Gly Ile His Lys Arg Gly Ala Glu Ile Asp	
		1010 1015 1020
50	Arg Leu Val Lys Gly Cys Thr Gly Val Arg Ala Thr Thr Gly Gln His	
		1025 1030 1035 1040
	Pro Gly Gly Ile Ile Val Val Pro Asp Tyr Met Asp Ile Tyr Asp Phe	
		1045 1050 1055
55	Thr Pro Ile Gln Tyr Pro Ala Asp Asp Gln Asn Ser Ala Trp Met Thr	
		1060 1065 1070
	Thr His Phe Asp Phe His Ser Ile His Asp Asn Val Leu Lys Leu Asp	
60		1075 1080 1085

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	Ile Leu Gly His Asp Asp Pro Thr Met Ile Arg Met Leu Gln Asp Leu	
	1090	1095 1100
5	Ser Gly Ile Asp Pro Lys Thr Ile Pro Val Asp Asp Lys Glu Val Met	
	1105	1110 1115 1120
	Gln Ile Phe Ser Thr Pro Glu Ser Leu Gly Val Thr Glu Asp Glu Ile	
		1125 1130 1135
10	Leu Cys Lys Thr Gly Thr Phe Gly Val Pro Asn Ser Asp Arg Ile Arg	
		1140 1145 1150
	Arg Gln Met Leu Glu Asp Thr Lys Pro Thr Thr Phe Ser Glu Leu Val	
15		1155 1160 1165
	Gln Ile Ser Gly Leu Ser His Gly Thr Asp Val Trp Leu Gly Asn Ala	
		1170 1175 1180
20	Gln Glu Leu Ile Lys Thr Gly Ile Cys Asp Leu Ser Ser Val Ile Gly	
		1185 1190 1195 1200
	Cys Arg Asp Asp Ile Met Val Tyr Leu Met Tyr Ala Gly Leu Glu Pro	
		1205 1210 1215
25	Ser Met Ala Phe Lys Ile Met Glu Ser Val Arg Lys Gly Lys Gly Leu	
		1220 1225 1230
	Thr Glu Glu Met Ile Glu Thr Met Lys Glu Asn Glu Val Pro Asp Trp	
30		1235 1240 1245
	Tyr Leu Asp Ser Cys Leu Lys Ile Lys Tyr Ile Phe Pro Lys Ala His	
		1250 1255 1260
35	Ala Ala Ala Tyr Val Leu Met Ala Val Arg Ile Ala Tyr Phe Lys Val	
		1265 1270 1275 1280
	His His Pro Leu Tyr Tyr Tyr Ala Ser Tyr Phe Thr Ile Arg Ala Ser	
		1285 1290 1295
40	Asp Phe Asp Leu Ile Thr Met Ile Lys Asp Lys Thr Ser Ile Arg Asn	
		1300 1305 1310
	Thr Val Lys Asp Met Tyr Ser Arg Tyr Met Asp Leu Gly Lys Lys Glu	
45		1315 1320 1325
	Lys Asp Val Leu Thr Val Leu Glu Ile Met Asn Glu Met Ala His Arg	
		1330 1335 1340
50	Gly Tyr Arg Met Gln Pro Ile Ser Leu Glu Lys Ser Gln Ala Phe Glu	
		1345 1350 1355 1360
	Phe Ile Ile Glu Gly Asp Thr Leu Ile Pro Pro Phe Ile Ser Val Pro	
		1365 1370 1375
55	Gly Leu Gly Glu Asn Val Ala Lys Arg Ile Val Glu Ala Arg Asp Asp	
		1380 1385 1390
60	Gly Pro Phe Leu Ser Lys Glu Asp Leu Asn Lys Lys Ala Gly Leu Tyr	
		1395 1400 1405

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Gln Lys Ile Ile Glu Tyr Leu Asp Glu Leu Gly Ser Leu Pro Asn Leu
 1410 1415 1420

5 Pro Asp Lys Ala Gln Leu Ser Ile Phe Asp Met
 1425 1430 1435

This invention also relates to the sequence of the *S. aureus* dnaN gene encoding the beta subunit. The nucleotide sequence is as follows (SEQ. ID. No.9):

10 ATGATGGAAT TCACTATTAA AAGAGATTAT TTTATTACAC AATTAAATGA CACATTAAAA 60
 GCTATTTTCAC CAAGAACAAC ATTACCTATA TTAAGTGGTA TCAAAATCGA TGCAGAAAGAA 120
 CATGAAGTTA TATTAAGTGG TTCAGACTCT GAAATTTCAA TAGAAATCAC TATTCCTAAA 180
 15 ACTGTAGATG GCGAAGATAT TGTCAATATT TCAGAAACAG GCTCAGTAGT ACTTCCTGGA 240
 CGATTCTTTG TTGATATTAT AAAAAAATTA CCTGGTAAAG ATGTTAAATT ATCTACAAAT 300
 20 GAACAATTCC AGACATTAAT TACATCAGGT CATTCTGAAT TTAATTTGAG TGGCTTAGAT 360
 CCAGATCAAT ATCCTTTATT ACCTCAAGTT TCTAGAGATG ACGCAATTCA ATTGTCGGTA 420
 AAAGTACTTA AAAACGTGAT TGCACAAACG AATTTTGAG TGTCCACCTC AGAAACACGC 480
 25 CCAGTACTAA CTGGTGTGAA CTGGCTTATA CAAGAAAATG AATTAATATG CACAGCGACT 540
 GATTACACCC GCTTGGCTGT AAGAAAGTTG CAGTTAGAAG ATGTTTCTGA AAACAAAAAT 600
 30 GTCATCATTC CAGGTAAGGC TTTAGCTGAA TTAAATAAAA TTATGTCTGA CAATGAAGAA 660
 GACATTGATA TCTTCTTTGC TTCAAACCAA GTTTTATTTA AAGTTGGAAA TGTGAACCTT 720
 ATTTCTCGAT TATTAGAAGG ACATTATCCT GATACAACAC GTTTATTCCC TGAAAACTAT 780
 35 GAAATTAAAT TAAGTATAGA CAATGGGGAG TTTTATCATG CGATTGATCG TGCCTCTTTA 840
 TTAGCACGTG AAGGTGGTAA TAACGTTATT AAATTAAGTA CAGGTGATGA CGTTGTTGAA 900
 40 TTATCTTCTA CATCACCAGA AATTGGTACT GTAAAAGAAG AAGTTGATGC AAACGATGTT 960
 GAAGGTGGTA GCCTGAAAAT TTCATTCAAC TCTAAATATA TGATGGATGC TTTAAAAGCA 1020
 ATCGATAATG ATGAGGTTGA AGTTGAATTC TTCGGTACAA TGAAACCATT TATTCTAAAA 1080
 45 CCAAAGGTG ACGACTCGGT AACGCAATTA ATTTTACCAA TCAGAACTTA CTAA 1134

This amino acid sequence of *S. aureus* beta subunit is as follows (SEQ. ID. No. 10):

50 Met Met Glu Phe Thr Ile Lys Arg Asp Tyr Phe Ile Thr Gln Leu Asn
 1 5 10 15

55 Asp Thr Leu Lys Ala Ile Ser Pro Arg Thr Thr Leu Pro Ile Leu Thr
 20 25 30

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	Gly	Ile	Lys	Ile	Asp	Ala	Lys	Glu	His	Glu	Val	Ile	Leu	Thr	Gly	Ser	
			35					40					45				
5	Asp	Ser	Glu	Ile	Ser	Ile	Glu	Ile	Thr	Ile	Pro	Lys	Thr	Val	Asp	Gly	
		50					55					60					
	Glu	Asp	Ile	Val	Asn	Ile	Ser	Glu	Thr	Gly	Ser	Val	Val	Leu	Pro	Gly	
10		65				70					75				80		
	Arg	Phe	Phe	Val	Asp	Ile	Ile	Lys	Lys	Leu	Pro	Gly	Lys	Asp	Val	Lys	
					85					90					95		
	Leu	Ser	Thr	Asn	Glu	Gln	Phe	Gln	Thr	Leu	Ile	Thr	Ser	Gly	His	Ser	
15				100					105					110			
	Glu	Phe	Asn	Leu	Ser	Gly	Leu	Asp	Pro	Asp	Gln	Tyr	Pro	Leu	Leu	Pro	
			115					120					125				
20	Gln	Val	Ser	Arg	Asp	Asp	Ala	Ile	Gln	Leu	Ser	Val	Lys	Val	Leu	Lys	
		130					135						140				
	Asn	Val	Ile	Ala	Gln	Thr	Asn	Phe	Ala	Val	Ser	Thr	Ser	Glu	Thr	Arg	
25		145				150					155					160	
	Pro	Val	Leu	Thr	Gly	Val	Asn	Trp	Leu	Ile	Gln	Glu	Asn	Glu	Leu	Ile	
					165				170						175		
	Cys	Thr	Ala	Thr	Asp	Ser	His	Arg	Leu	Ala	Val	Arg	Lys	Leu	Gln	Leu	
30				180					185					190			
	Glu	Asp	Val	Ser	Glu	Asn	Lys	Asn	Val	Ile	Ile	Pro	Gly	Lys	Ala	Leu	
			195					200					205				
35	Ala	Glu	Leu	Asn	Lys	Ile	Met	Ser	Asp	Asn	Glu	Glu	Asp	Ile	Asp	Ile	
			210				215						220				
	Phe	Phe	Ala	Ser	Asn	Gln	Val	Leu	Phe	Lys	Val	Gly	Asn	Val	Asn	Phe	
40		225				230					235					240	
	Ile	Ser	Arg	Leu	Leu	Glu	Gly	His	Tyr	Pro	Asp	Thr	Thr	Arg	Leu	Phe	
				245						250					255		
	Pro	Glu	Asn	Tyr	Glu	Ile	Lys	Leu	Ser	Ile	Asp	Asn	Gly	Glu	Phe	Tyr	
45				260				265						270			
	His	Ala	Ile	Asp	Arg	Ala	Ser	Leu	Leu	Ala	Arg	Glu	Gly	Gly	Asn	Asn	
			275					280					285				
50	Val	Ile	Lys	Leu	Ser	Thr	Gly	Asp	Asp	Val	Val	Glu	Leu	Ser	Ser	Thr	
			290				295					300					
	Ser	Pro	Glu	Ile	Gly	Thr	Val	Lys	Glu	Glu	Val	Asp	Ala	Asn	Asp	Val	
55		305				310					315				320		
	Glu	Gly	Gly	Ser	Leu	Lys	Ile	Ser	Phe	Asn	Ser	Lys	Tyr	Met	Met	Asp	
				325						330					335		
60	Ala	Leu	Lys	Ala	Ile	Asp	Asn	Asp	Glu	Val	Glu	Val	Glu	Phe	Phe	Gly	
				340				345						350			

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Thr Met Lys Pro Phe Ile Leu Lys Pro Lys Gly Asp Asp Ser Val Thr
 355 360 365

5 Gln Leu Ile Leu Pro Ile Arg Thr Tyr
 370 375

This invention also relates to the sequence of the *S. aureus* dnaG gene encoding a primase. The nucleotide sequence is as follows (SEQ. ID. No. 11):

10	ATGATAGGTT TGTGTCCTTT TCATGATGAA AAGACACCTT CATTACAGT TTCTGAAGAT	60
	AAACAAATCT GTCATTGTTT TGGTTGTAAG AAAGGTGGCA ATGTTTTTCA ATTTACTCAA	120
	GAAATTAAAG ACATATCATT TGTTGAAGCG GTTAAAGAAT TAGGTGATAG AGTTAATGTT	180
15	GCTGTAGATA TTGAGGCAAC ACAATCTAAC TCAAATGTTC AAATTGCTTC TGATGATTAA	240
	CAAATGATTG AAATGCATGA GTTAATACAA GAATTTTATT ATTACGCTTT AACAAAGACA	300
20	GTCGAAGGCG AACAAGCATT AACATACTTA CAAGAACGTG GTTTTACAGA TCGCGTTATT	360
	AAAGAGCGAG GCATTGGCTT TGCACCCGAT AGCTCACATT TTTGTCATGA TTTTCTTCAA	420
	AAAAAGGGTT ACGATATTGA ATTAGCATAT GAAGCCGGAT TATTATCACG TAACGAAGAA	480
25	AATTTCAAGT ATTACGATAG ATTTGGAAT CGTATTATGT TTCCTTTGAA AAATGCGCAA	540
	GGAAGAATTG TTGGATATTC AGGTCGAACA TATACCGGTC AAGAACCAAA ATACCTAAAT	600
30	AGTCTGAAA CGCCTATCTT TCAAAAAAGA AAGTTGTTAT ATAAGTTAGA TAAAGCAGCT	660
	AAATCAATTA GAAAATTAGA TGAAATTGTA TTAGTAGAAG GTTTTATGGA TGTATATAAA	720
	TCTGATACTG CTGGCTTGAA AAACGTTGTT GCAACAATGG GTACACAGTT GTCAGATGAA	780
35	CATATTACCT TTATACGAAA GTTAACATCA AATATAACAT TAATGTTTGA TGGGGATTTT	840
	GCGGGTAGTG AAGCAACACT TAAACAGGT CAACATTTGT TACAGCAAGG GCTAAATGTA	900
40	TTTGTATAC AATTGCCATC TGGCATGGAT CCGGATGAAT ACATTGGTAA GTATGGCAAC	960
	GACGCATTTA CTACTTTTGT AAAAAATGAC AAAAAGTCAT TTGCACATTA TAAAGTAAGT	1020
	ATATTAAGAG ATGAAATTGC ACATAATGAC CTTTCATATG AACGTTATTT GAAAGAACTG	1080
45	AGTCATGACA TTTCATTTAT GAAGTCATCA ATTCTGCAAC AAAAGGCTAT AAATGATGTT	1140
	GCGCCATTTT TCAATGTTAG TCCTGAGCAG TTAGCTAACG AAATACAATT CAATCAAGCA	1200
50	CCAGCCAATT ATTATCCAGA AGATGAGTAT GGCGGTTATG ATGAGTATGG CGGTTATATT	1260
	GAACCTGAGC CAATTGGTAT GGCACAATTT GACAATTTGA GCCGTCGAGA AAAAGCGGAG	1320
	CGAGCATTTT TAAACATTT AATGAGAGAT AAAGATACAT TTTTAAATTA TTATGAAAGT	1380
55	GTTGATAAGG ATAACTTCAC AAATCAGCAT TTAAATATG TATTCGAAGT CTTACATGAT	1440
	TTTTATGCGG AAAATGATCA ATATAATATC AGTGATGCTG TGCAGTATGT TAATTCAAAT	1500

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GAGTTGAGAG AAACACTAAT TAGCTTAGAA CAATATAATT TGAATGGCGA ACCATATGAA 1560
AATGAAATTG ATGATTATGT CAATGTTATT AATGAAAAAG GACAAGAAAC AATTGAGTCA 1620
5 TTGAATCATA AATTAAGGGA AGCTACAAGG ATTGGCGATG TAGAATTACA AAAATACTAT 1680
TTACAGCAAA TTGTTGCTAA GAATAAGAA CGCATGTAG 1719

The amino acid sequence of primase encoded by *S. aureus* dnaG is as
10 follows (SEQ. ID. No. 12):

	Met	Ile	Gly	Leu	Cys	Pro	Phe	His	Asp	Glu	Lys	Thr	Pro	Ser	Phe	Thr	
	1				5					10					15		
15	Val	Ser	Glu	Asp	Lys	Gln	Ile	Cys	His	Cys	Phe	Gly	Cys	Lys	Lys	Gly	
				20				25					30				
	Gly	Asn	Val	Phe	Gln	Phe	Thr	Gln	Glu	Ile	Lys	Asp	Ile	Ser	Phe	Val	
20				35				40					45				
	Glu	Ala	Val	Lys	Glu	Leu	Gly	Asp	Arg	Val	Asn	Val	Ala	Val	Asp	Ile	
				50			55					60					
25	Glu	Ala	Thr	Gln	Ser	Asn	Ser	Asn	Val	Gln	Ile	Ala	Ser	Asp	Asp	Leu	
	65					70				75					80		
	Gln	Met	Ile	Glu	Met	His	Glu	Leu	Ile	Gln	Glu	Phe	Tyr	Tyr	Tyr	Ala	
				85				90							95		
30	Leu	Thr	Lys	Thr	Val	Glu	Gly	Glu	Gln	Ala	Leu	Thr	Tyr	Leu	Gln	Glu	
				100				105						110			
	Arg	Gly	Phe	Thr	Asp	Ala	Leu	Ile	Lys	Glu	Arg	Gly	Ile	Gly	Phe	Ala	
35				115				120					125				
	Pro	Asp	Ser	Ser	His	Phe	Cys	His	Asp	Phe	Leu	Gln	Lys	Lys	Gly	Tyr	
				130			135					140					
40	Asp	Ile	Glu	Leu	Ala	Tyr	Glu	Ala	Gly	Leu	Leu	Ser	Arg	Asn	Glu	Glu	
	145				150					155					160		
	Asn	Phe	Ser	Tyr	Tyr	Asp	Arg	Phe	Arg	Asn	Arg	Ile	Met	Phe	Pro	Leu	
				165				170						175			
45	Lys	Asn	Ala	Gln	Gly	Arg	Ile	Val	Gly	Tyr	Ser	Gly	Arg	Thr	Tyr	Thr	
				180				185						190			
	Gly	Gln	Glu	Pro	Lys	Tyr	Leu	Asn	Ser	Pro	Glu	Thr	Pro	Ile	Phe	Gln	
50				195			200						205				
	Lys	Arg	Lys	Leu	Leu	Tyr	Asn	Leu	Asp	Lys	Ala	Arg	Lys	Ser	Ile	Arg	
		210				215					220						
55	Lys	Leu	Asp	Glu	Ile	Val	Leu	Leu	Glu	Gly	Phe	Met	Asp	Val	Ile	Lys	
	225				230					235				240			
	Ser	Asp	Thr	Ala	Gly	Leu	Lys	Asn	Val	Val	Ala	Thr	Met	Gly	Thr	Gln	
				245				250						255			

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	Leu Ser Asp Glu His Ile Thr Phe Ile Arg Lys Leu Thr Ser Asn Ile	
	260	265 270
5	Thr Leu Met Phe Asp Gly Asp Phe Ala Gly Ser Glu Ala Thr Leu Lys	
	275	280 285
	Thr Gly Gln His Leu Leu Gln Gln Gly Leu Asn Val Phe Val Ile Gln	
10	290	295 300
	Leu Pro Ser Gly Met Asp Pro Asp Glu Tyr Ile Gly Lys Tyr Gly Asn	
	305	310 315 320
15	Asp Ala Phe Thr Thr Phe Val Lys Asn Asp Lys Lys Ser Phe Ala His	
	325	330 335
	Tyr Lys Val Ser Ile Leu Lys Asp Glu Ile Ala His Asn Asp Leu Ser	
	340	345 350
20	Tyr Glu Arg Tyr Leu Lys Glu Leu Ser His Asp Ile Ser Leu Met Lys	
	355	360 365
	Ser Ser Ile Leu Gln Gln Lys Ala Ile Asn Asp Val Ala Pro Phe Phe	
25	370	375 380
	Asn Val Ser Pro Glu Gln Leu Ala Asn Glu Ile Gln Phe Asn Gln Ala	
	385	390 395 400
30	Pro Ala Asn Tyr Tyr Pro Glu Asp Glu Tyr Gly Gly Tyr Asp Glu Tyr	
	405	410 415
	Gly Gly Tyr Ile Glu Pro Glu Pro Ile Gly Met Ala Gln Phe Asp Asn	
	420	425 430
35	Leu Ser Arg Arg Glu Lys Ala Glu Arg Ala Phe Leu Lys His Leu Met	
	435	440 445
	Arg Asp Lys Asp Thr Phe Leu Asn Tyr Tyr Glu Ser Val Asp Lys Asp	
40	450	455 460
	Asn Phe Thr Asn Gln His Phe Lys Tyr Val Phe Glu Val Leu His Asp	
	465	470 475 480
45	Phe Tyr Ala Glu Asn Asp Gln Tyr Asn Ile Ser Asp Ala Val Gln Tyr	
	485	490 495
	Val Asn Ser Asn Glu Leu Arg Glu Thr Leu Ile Ser Leu Glu Gln Tyr	
	500	505 510
50	Asn Leu Asn Gly Glu Pro Tyr Glu Asn Glu Ile Asp Asp Tyr Val Asn	
	515	520 525
	Val Ile Asn Glu Lys Gly Gln Glu Thr Ile Glu Ser Leu Asn His Lys	
55	530	535 540
	Leu Arg Glu Ala Thr Arg Ile Gly Asp Val Glu Leu Gln Lys Tyr Tyr	
	545	550 555 560
60	Leu Gln Gln Ile Val Ala Lys Asn Lys Glu Arg Met	
	565	570

Fragments of the above polypeptides or proteins are also encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding the protein of the present invention are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for activity according to the procedures described below.

As an alternative, fragments of replication proteins can be produced by digestion of a full-length replication protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave replication proteins at different sites based on the amino acid sequence of the protein. Some of the fragments that result from proteolysis may be active.

In another approach, based on knowledge of the primary structure of the protein, fragments of a replication protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for increased expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences of replication proteins being produced. Alternatively, subjecting a full length replication protein to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydrophatic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which cotranslationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of 50 continuous bases of SEQ. ID. Nos. 1, 3, 5, 7, 9, or 11 under stringent conditions such as those characterized by a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C and remaining bound when subject to washing the SSC buffer at a temperature of 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

The proteins or polypeptides of the present invention are preferably produced in purified form (preferably at least 80%, more preferably 90%, pure) by conventional techniques. Typically, the proteins or polypeptides of the present invention is secreted into the growth medium of recombinant host cells. Alternatively, the proteins or polypeptides of the present invention are produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to purification procedures such as ammonium sulfate precipitation, gel filtration, ion exchange chromatography, FPLC, and HPLC.

The DNA molecule encoding replication polypeptides or proteins derived from Gram positive bacteria can be incorporated in cells using conventional recombinant DNA technology. Generally, this involved inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral
5 vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see
10 F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard
15 cloning procedures in the art, as described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host
20 cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression
25 elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

30 Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from

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those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further procaryotic promoters are not recognized and do not function in eucaryotic cells.

5 Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short
10 nucleotide sequence of mRNA that is located before the same codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct
15 positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

 Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of
20 suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promotor, *trp* promotor, *recA* promotor, ribosomal RNA promotor, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA
25 segments. Additionally, a hybrid *trp-lacUV5 (tac)* promotor or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

 Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations,
30 the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc.,

are under different controls. Additionally, the cell may carry the gene for a heterologous RNA polymerase such as from phage T7. Thus, a promoter specific for T7 RNA polymerase is used. The T7 RNA polymerase may be under inducible control.

5 Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in 10 *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, an SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage 15 lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding a replication polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a 20 host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, viruses, yeast, mammalian cells, insects, plants, and the like.

The invention provides efficient methods of identifying 25 pharmacological agents or lead compounds for agents active at the level of a replication protein function, particularly DNA replication. Generally, these screening methods involve assaying for compounds which interfere with the replication activity. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the 30 pharmaceutical industries for animal and human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development. Target therapeutic indications are

limited only in that the target cellular function be subject to modulation, usually inhibition, by disruption of a replication activity or the formation of a complex comprising a replication protein and one or more natural intracellular binding targets. Target indications may include arresting cell growth or causing cell death resulting in recovery from the bacterial infection in animal studies.

A wide variety of assays for activity and binding agents are provided, including DNA synthesis, ATPase, clamp loading onto DNA, protein-protein binding assays, immunoassays, cell based assays, etc. The replication protein compositions, used to identify pharmacological agents, are in isolated, partially pure or pure form and are typically recombinantly produced. The replication protein may be part of a fusion product with another peptide or polypeptide (e.g., a polypeptide that is capable of providing or enhancing protein-protein binding, stability under assay conditions (e.g., a tag for detection or anchoring), etc.). The assay mixtures comprise a natural intracellular replication protein binding target such as DNA, another protein, NTP, or dNTP. For binding assays, while native binding targets may be used, it is frequently preferred to use portions (e.g., peptides, nucleic acid fragments) thereof so long as the portion provides binding affinity and avidity to the subject replication protein conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Generally, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control (i.e. at zero concentration or below the limits of assay detection). Additional controls are often present such as a positive control, a dose response curve, use of known inhibitors, use of control heterologous proteins, etc. Candidate agents encompass numerous chemical classes, though typically they are organic compounds; preferably they are small organic compounds and are obtained from a wide variety of sources, including libraries of synthetic or natural compounds. A variety of other reagents may also be included in the mixture. These include reagents like salts, buffers, neutral proteins (e.g., albumin, detergents, etc.), which may be used to facilitate optimal binding and/or reduce nonspecific or background interactions, etc. Also reagents that otherwise improve the efficiency of the assay (e.g., protease inhibitors, nuclease inhibitors, antimicrobial agents, etc.) may be used.

Generally, replication protein-specificity of the binding agent is shown by binding equilibrium constants. Such agents are capable of selectively binding a replication protein (i.e., with an equilibrium constant at least about 10^7 M^{-1} , preferably, at least about 10^8 M^{-1} , more preferably, at least about 10^9 M^{-1}). A wide variety of cell-based and cell-free assays may be used to demonstrate replication protein-specific activity, binding, gel shift assays, immunoassays, etc.

The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the replication protein specifically binds the cellular binding target, portion, or analog. The mixture of components can be added in any order that provides for the requisite bindings. Incubations may be performed at any temperature which facilitates optimal binding, typically between 4 and 40°C, more commonly between 15° and 40°C. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening, and are typically between 0.1 and 10 hours, preferably less than 5 hours, more preferably less than 2 hours.

After incubation, the presence or absence of activity or specific binding between the replication protein and one or more binding targets is detected by any convenient way. For cell-free activity and binding type assays, a separation step may be used to separate the activity product or the bound from unbound components. Separation may be effected by precipitation (e.g., immunoprecipitation), immobilization (e.g., on a solid substrate such as a microtiter plate), etc., followed by washing. Many assays that do not require separation are also possible such as use of europium conjugation in proximity assays or a detection system that is dependent on a product or loss of substrate.

Detection may be effected in any convenient way. For cell-free activity and binding assays, one of the components usually comprises or is coupled to a label. A wide variety of labels may be employed – essentially any label that provides for detection of DNA product, loss of DNA substrate, conversion of a nucleotide substrate, or bound protein is useful. The label may provide for direct detection such as radioactivity, fluorescence, luminescence, optical, or electron density, etc. or indirect detection such as an epitope tag, an enzyme, etc. The label may be appended to the protein (e.g., a phosphate group comprising a radioactive isotope of phosphorous), or incorporated into the DNA substrate or the protein structure (e.g., a methionine residue comprising a radioactive isotope of sulfur.) A variety of methods may be used to detect the label depending on the nature of the label and other assay components. For example, the label may be detected bound to the solid substrate, or a portion of the bound complex containing the label may be separated from the solid substrate, and thereafter the label detected. Labels may be directly detected through optical or electron density, radioactive emissions, nonradiative energy transfer, fluorescence emission, etc. or indirectly detected with antibody conjugates, etc. For example, in the case of radioactive labels, emissions may be detected directly (e.g., with particle counters) or indirectly (e.g., with scintillation cocktails and counters).

The present invention identifies the type of replication system that Gram positive bacteria utilize. Specifically, the replicase is comprised of a DNA polymerase III-type enzyme and it is made functional by other components that are needed for processive function. These components include a sliding clamp and a clamp loader. Hence, Gram negative bacteria do not utilize the replication strategies exemplified by one and two component processive replicases.

The present invention also identifies, partially purifies, and characterizes a second Pol III-type replicase. The polymerase of the second Pol III-type enzyme, termed Pol III-2, behaves like Pol III-L in that it also functions with the clamp and clamp loader components.

This invention also expresses and purifies a protein from a Gram positive bacteria that is homologous to the *E. coli* beta subunit. The invention demonstrates that it behaves like a circular protein. Further, this invention shows that

the beta subunit from a Gram positive bacteria is functional with both Pol III-L from a Gram positive bacteria and with DNA polymerase III from a Gram negative bacteria. This result can be explained by an interaction between the clamp and the polymerase that has been conserved during the evolutionary divergence of Gram positive and

5 Gram negative cells. A chemical inhibitor that would disrupt this interaction would be predicted to have a broad spectrum of antibiotic activity, shutting down replication in gram negative and gram positive cells alike. This assay, and others based on this interaction, can be devised to screen chemicals for such inhibition. Further, since all the proteins in this assay are highly overexpressed through recombinant techniques,

10 sufficient quantities of the protein reagents can be obtained for screening hundreds of thousands of compounds.

The present invention provides methods by which replication proteins from a Gram positive bacteria are used to discover new pharmaceutical agents. The function of replication proteins is quantified in the presence of different chemical

15 compounds. A chemical compound that inhibits the function is a candidate antibiotic. Some replication proteins from a Gram positive bacteria and from a Gram negative bacteria can be interchanged for one another. Hence, they can function as mixtures. Reactions that assay for the function of enzyme mixtures consisting of proteins from Gram positive bacteria and from Gram negative bacteria can also be used to discover

20 drugs. Suitable *E. coli* replication proteins are the subunits of its Pol III holoenzyme which are described in U.S. Patent Nos. 5,583,026 and 5,668,004, which are hereby incorporated by reference.

The methods described here to obtain genes, and the assays demonstrating activity behavior of *S. aureus* are likely to generalize to all members of

25 the *Staphylococcus* genus and to all Gram positive bacteria.

The present invention describes a method to identify chemicals that inhibit the activity of the Pol III-2 and/or Pol III-L. This method involves contacting primed DNA with the DNA polymerase in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The

30 reaction mixture is subjected to conditions effective to achieve nucleic acid polymerization in the absence of the candidate pharmaceutical and the presence or

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absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product.

The present invention describes a method to identify chemicals that inhibit the ability of a beta subunit to stimulate Pol III-2 and/or Pol III-L. This method involves contacting a linear primed DNA with a beta subunit and a DNA polymerase in the presence of the candidate compound, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions which, in the absence of the candidate compound, would affect nucleic acid polymerization and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product. The beta subunit and/or the DNA polymerase are derived from a Gram positive bacterium.

The present invention also describes a method to identify candidate pharmaceuticals that inhibit the activity of a gamma complex (or a subunit or subassembly of the gamma complex) and a beta subunit in stimulating either Pol III-2 or Pol III-L. The method includes contacting a primed DNA (which may be coated with SSB) with a DNA polymerase, a beta subunit, and a gamma complex (or subunit or subassembly of the gamma complex) in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions which in the absence of the candidate pharmaceutical would effect nucleic acid polymerization and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product. The DNA polymerase, the beta subunit, and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a beta subunit and a DNA polymerase to interact physically. This method involves contacting the beta subunit with the DNA polymerase in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the DNA polymerase and the beta subunit would interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the beta unit and the DNA

polymerase. The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and the DNA polymerase. The DNA polymerase and/or the beta subunit are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that
5 inhibit the ability of a beta subunit and a gamma complex (or a subunit or subassembly of the gamma complex) to interact. This method includes contacting the beta subunit with the gamma complex (or subunit or subassembly of the gamma complex) in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the gamma complex (or
10 the subunit or subassembly of the gamma complex) and the beta subunit would interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the beta subunit and the gamma complex (or the subunit or subassembly of the gamma complex). The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and the gamma
15 complex (or the subunit or subassembly of the gamma complex) . The beta subunit and/or the gamma complex or subunit thereof is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a gamma complex (or a subassembly of the gamma complex) to
20 assemble a beta subunit onto a DNA molecule. This method involves contacting a circular primed DNA molecule (which may be coated with SSB) with the gamma complex (or the subassembly thereof) and the beta subunit in the presence of the candidate pharmaceutical, and ATP or dATP to form a reaction mixture. The reaction mixture is subjected to conditions under which the gamma complex (or subassembly)
25 assembles the beta subunit on the DNA molecule absent the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of the beta subunit on the DNA molecule. The beta subunit and/or the gamma complex are derived from a Gram positive bacterium.

30 The present invention describes a method to identify chemicals that inhibit the ability of a gamma complex (or a subunit(s) of the gamma complex) to disassemble a beta subunit from a DNA molecule. This method comprises contacting

a DNA molecule onto which the beta subunit has been assembled in the presence of the candidate pharmaceutical, to form a reaction mixture. The reaction mixture is subjected to conditions under which the gamma complex (or a subunit(s) or subassembly of the gamma complex) disassembles the beta subunit from the DNA molecule absent the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the presence of the beta subunit on the DNA molecule. The beta subunit and/or the gamma complex are derived from a Gram positive bacterium.

10 The present invention describes a method to identify chemicals that disassemble a beta subunit from a DNA molecule. This method involves contacting a circular primed DNA molecule (which may be coated with SSB) upon which the beta subunit has been assembled (e.g., by action of the gamma complex) with the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of the beta subunit on the DNA molecule. The beta subunit is derived from a Gram positive bacterium.

 The present invention describes a method to identify chemicals that inhibit the dATP/ATP binding activity of a gamma complex or a gamma complex subunit (e.g., gamma subunit). This method includes contacting the gamma complex (or the gamma complex subunit) with dATP/ATP either in the presence or absence of a DNA molecule and/or the beta subunit in the presence of the candidate pharmaceutical to form a reaction. The reaction mixture is subjected to conditions in which the gamma complex (or the subunit of gamma complex) interacts with dATP/ATP in the absence of the candidate pharmaceutical. The reaction is analyzed to determine if dATP/ATP is bound to the gamma complex (or the subunit of gamma complex) in the presence of the candidate pharmaceutical. The candidate pharmaceutical is detected by the absence of hydrolysis. The gamma complex and/or the beta subunit is derived from a Gram positive bacterium.

30 The present invention describes a method to identify chemicals that inhibit the dATP/ATPase activity of a gamma complex or a gamma complex subunit (e.g., the gamma subunit). This method involves contacting the gamma complex (or

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the gamma complex subunit) with dATP/ATP either in the presence or absence of a DNA molecule and/or a beta subunit in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions in which the gamma subunit (or complex) hydrolyzes dATP/ATP in the absence of the
5 candidate pharmaceutical. The reaction is analyzed to determine if dATP/ATP was hydrolyzed. Suitable candidate pharmaceuticals are identified by the absence of hydrolysis. The gamma complex and/or the beta subunit is derived from a Gram positive bacterium.

The present invention describes methods to identify chemicals that
10 inhibit the activity of a DNA polymerase encoded by either the dnaE gene or PolC gene. These methods are as follows.

1) Contacting a primed DNA molecule with the encoded product of the dnaE gene or PolC gene in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is
15 subjected to conditions, which in the absence of the candidate pharmaceutical, affect nucleic acid polymerization and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of extension product. The protein encoded by the dnaE gene and PolC gene is derived from a Gram positive bacterium.

2) Contacting a linear primed DNA molecule with a beta subunit and the encoded product of dnaE or PolC in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions, which in the absence of the candidate
25 pharmaceutical, affect nucleic acid polymerization, and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of extension product. The protein encoded by the dnaE gene and PolC gene is derived from a Gram positive bacterium.

3) Contacting a circular primed DNA molecule (may be coated with SSB) with a gamma complex, a beta subunit and the encoded product of a dnaE
30 gene or PolC gene in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions, which in the absence of the candidate pharmaceutical, affect nucleic acid

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polymerization, and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product. The protein encoded by the dnaE gene and PolC gene, the beta subunit, and/or the gamma complex are derived from a Gram positive bacterium.

- 5 4) Contacting a beta subunit with the product encoded by a dnaE gene or PolC gene in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is then analyzed for interaction between the beta subunit and the product encoded by the dnaE gene or PolC gene. The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and
10 the product encoded by the dnaE gene or PolC gene. The beta subunit and/or the protein encoded by the dnaE gene and PolC gene is derived from a Gram positive bacterium.

The present invention discloses a method to identify chemicals that inhibit a DnaB helicase. The method includes contacting the DnaB helicase with a
15 DNA molecule substrate that has a duplex region in the presence of a nucleoside or deoxynucleoside triphosphate energy source and a candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support helicase activity in the absence of the candidate pharmaceutical. The DNA duplex molecule in the reaction mixture is analyzed for whether it is converted to ssDNA. The candidate
20 pharmaceutical is detected by the absence of conversion of the duplex DNA molecule to the ssDNA molecule. The DnaB helicase is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the nucleoside or deoxynucleoside triphosphatase activity of a DnaB helicase.
25 The method includes contacting the DnaB helicase with a DNA molecule substrate that has a duplex region in the presence of a nucleoside or deoxynucleoside triphosphate energy source and the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support nucleoside or deoxynucleoside triphosphatase activity of the DnaB helicase in the absence of the
30 candidate pharmaceutical. The candidate pharmaceutical is detected by the absence of conversion of nucleoside or deoxynucleoside triphosphate to nucleoside or

deoxynucleoside diphosphate. The DnaB helicase is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit a primase. The method includes contacting primase with a ssDNA molecule in the presence of a candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support primase activity (e.g., the presence of nucleoside or deoxynucleoside triphosphates, appropriate buffer, presence or absence of DnaB protein) in the absence of the candidate pharmaceutical. Suitable candidate pharmaceuticals are identified by the absence of primer formation detected either directly or indirectly. The primase is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a primase and the protein encoded by a DnaB gene to interact. This method includes contacting the primase with the protein encoded by the DnaB gene in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the primase and the protein encoded by the DnaB gene interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the primase and the protein encoded by the DnaB gene. The candidate pharmaceutical is detected by the absence of interaction between the primase and the protein encoded by the DnaB gene. The primase and/or the DnaB gene are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a protein encoded by a DnaB gene to interact with a DNA molecule. This method includes contacting the protein encoded by the DnaB gene with the DNA molecule in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the DNA molecule and the protein encoded by the DnaB gene interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the protein encoded by the DnaB gene and the DNA molecule. The candidate pharmaceutical is detected by the absence of interaction between the DNA molecule and the protein encoded by the DnaB gene. The DnaB gene is derived from a Gram positive bacterium.

EXAMPLES

Example 1 - Materials

5 Labeled deoxy- and ribonucleoside triphosphates were from Dupont-New England Nuclear; unlabelled deoxy- and ribonucleoside triphosphates were from Pharmacia-LKB; *E. coli* replication proteins were purified as described, alpha, epsilon, gamma, and tau (Studwell, et al., "Processive Replication is Contingent on the Exonuclease Subunit of DNA Polymerase III Holoenzyme," J. Biol. Chem.,
10 265:1171-1178 (1990), which is hereby incorporated by reference), beta (Kong, et. al., "Three Dimensional Structure of the Beta Subunit of *Escherichia coli* DNA Polymerase III Holoenzyme: A Sliding DNA Clamp," Cell, 69:425-437 (1992), which is hereby incorporated by reference), delta and delta prime (Dong, et. al., "DNA Polymerase III Accessory Proteins. I. *HolA* and *holB* Encoding δ and δ' ," J.
15 Biol. Chem., 268:11758-11765 (1993), which is hereby incorporated by reference), chi and psi (Xiao, et. al., "DNA Polymerase III Accessory Proteins. III. *HolC* and *holD* Encoding chi and psi," J. Biol. Chem., 268:11773-11778 (1993), which is hereby incorporated by reference), theta (Studwell-Vaughan, et al., "DNA Polymerase III Accessory Proteins. V. Theta Encoded by *holE*," J. Biol. Chem.,
20 268:11785-11791 (1993), which is hereby incorporated by reference), and SSB (Weiner, et. al., "The Deoxyribonucleic Acid Unwinding Protein of *Escherichia coli*," J. Biol. Chem., 250:1972-1980 (1975), which is hereby incorporated by reference). *E. coli* Pol III core, and gamma complex (composed of subunits: gamma, delta, delta prime, chi, and psi) were reconstituted as described in Onrust, et. al., "Assembly of a
25 Chromosomal Replication Machine: Two DNA Polymerases, a Clamp Loader and Sliding Clamps in One Holoenzyme Particle. I. Organization of the Clamp Loader," J. Biol. Chem., 270:13348-13357 (1995), which is hereby incorporated by reference. Pol III* was reconstituted and purified as described in Onrust, et. al., "Assembly of a Chromosomal Replication Machine: Two DNA Polymerases, a Clamp Loader and
30 Sliding Clamps in One Holoenzyme Particle. III. Interface Between Two Polymerases and the Clamp Loader," J. Biol. Chem., 270:13366-13377 (1995), which is hereby incorporated by reference. Protein concentrations were quantitated by the Protein Assay (Bio-Rad) method using bovine serum albumin (BSA) as a standard.

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DNA oligonucleotides were synthesized by Oligos etc. Calf thymus DNA was from Sigma. Buffer A is 20 mM Tris-HCl (pH=7.5), 0.5 mM EDTA, 2 mM DTT, and 20% glycerol. Replication buffer is 20 mM Tris-Cl (pH 7.5), 8 mM MgCl₂, 5 mM DTT, 0.5 mM EDTA, 40 µg/ml BSA, 4% glycerol, 0.5 mM ATP, 3 mM each dCTP, dGTP, dATP, and 20 µM [α -³²P]dTTP. P-cell buffer was 50 mM potassium phosphate (pH 7.6), 5 mM DTT, 0.3 mM EDTA, 20% glycerol. T.E. buffer is 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. Cell lysis buffer was 50 mM Tris-HCl (pH 8.0) 10 % sucrose, 1 M NaCl, 0.3 mM spermidine.

10 **Example 2 - Calf Thymus DNA Replication Assays**

These assays were used in the purification of DNA polymerases from *S. aureus* cell extracts. Assays contained 2.5 µg activated calf thymus DNA in a final volume of 25 µl replication buffer. An aliquot of the fraction to be assayed was added to the assay mixture on ice followed by incubation at 37°C for 5 min. DNA synthesis was quantitated using DE81 paper as described in Rowen, et al., "Primase, the DnaG Protein of *Escherichia coli*. An Enzyme Which Starts DNA Chains," J. Biol. Chem., 253:758-764 (1979), which is hereby incorporated by reference.

20 **Example 3 - PolydA-oligodT Replication Assays**

PolydA-oligodT was prepared as follows. PolydA of average length 4500 nucleotides was purchased from SuperTecs. OligodT35 was synthesized by Oligos etc. 145 µl of 5.2 mM (as nucleotide) polydA and 22 µl of 1.75 mM (as nucleotide) oligodT were mixed in a final volume of 2100 µl T.E. buffer (ratio as nucleotide was 21:1 polydA to oligodT). The mixture was heated to boiling in a 1 ml eppendorf tube, then removed and allowed to cool to room temperature. Assays were performed in a final volume of 25 µl 20 mM Tris-Cl (pH 7.5), 8 mM MgCl₂, 5 mM DTT, 0.5 mM EDTA, 40 µg/ml BSA, 4% glycerol, containing 20 µM [α -³²P]dTTP and 0.36 µg polydA-oligodT. Proteins were added to the reaction on ice, then shifted to 37°C for 5 min. DNA synthesis was quantitated using DE81 paper as described in Rowen, et al., "Primase, the DnaG Protein of *Escherichia coli*. An Enzyme Which

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Two strains of *S. aureus* were used. For PCR of the first fragment of the dnaX gene sequence, the strain was ATCC 25923. For all other work the strain was strain 4220 (a gift of Dr. Pat Schlievert, University of Minnesota). This strain lacks a gene needed for producing toxic shock (Kreiswirth, et al., "The Toxic Shock Syndrome Exotoxin Structural Gene is Not Detectably Transmitted by a Prophage," Nature, 305:709-712 (1996) and Balan, et al., "Autocrine Regulation of Toxin Synthesis by *Staphylococcus aureus*," Proc. Natl. Acad. Sci. USA, 92:1619-1623 (1995), which are hereby incorporated by reference). *S. aureus* cells were grown overnight at 37°C in LB containing 0.5% glucose. Cells were collected by centrifugation (24 g wet weight). Cells were resuspended in 80 ml solution I (50 mM

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glucose, 10 mM EDTA, 25 mM Tris-HCL (pH 8.0)). Then, SDS and NaOH were added to 1% and 0.2 N, respectively, followed by incubation at 65°C for 30 min. to lyse the cells. 68.5 ml of 3 M sodium acetate (pH 5.0) was added followed by centrifugation at 12,000 rpm for 30 min. The supernatant was discarded and the pellet was washed twice with 50 ml of 6M urea, 10 mM Tris-HCL (pH 7.5), 1 mM EDTA) using a dounce homogenizer. After each wash, the resuspended pellet was collected by centrifugation (12,000 rpm for 20 min.). After the second wash, the pellet was resuspended in 50 ml 10 mM T.E. buffer using a dounce homogenizer and then incubated for 30 min. at 65°C. The solution was centrifuged at 12,000 rpm for 20 min., and the viscous supernatant was collected. 43.46 g CsCl₂ was added to the 50 ml of supernatant (density between 1.395-1.398) and poured into two 35 ml quick seal ultracentrifuge tubes (tubes were completely filled using the same density of CsCl₂ in T.E.). To each tube was added 0.5 ml of a 10 mg/ml stock of ethidium bromide. Tubes were spun at 55,000 rpm for 18 h at 18°C in a Sorvall TV860 rotor. The band of genomic DNA was extracted using a syringe and needle. Ethidium bromide was removed using two butanol extractions and then dialyzed against 4 l of T.E. at pH 8.0 overnight. The DNA was recovered by ethanol precipitation and then resuspended in T.E. buffer (1.7 mg total) and stored at -20°C.

20 **Example 6 - Cloning and Purification of *S. aureus* Pol III-L Holoenzyme**

To further characterize the mechanism of DNA replication in *S. aureus*, large amounts of its replication proteins were produced through use of the genes. The PolC gene encoding *S. aureus* Pol III-L holoenzyme has been sequenced and expressed in *E. coli* (Pacitti, et. al., "Characterization and Overexpression of the Gene Encoding *Staphylococcus aureus* DNA Polymerase III," *Gene*, 165:51-56 (1995), which is hereby incorporated by reference). The previous work utilized a pBS[KS] vector for expression in which the *E. coli* RNA polymerase is used for gene transcription. In the earlier study, the *S. aureus* Pol III gene was precisely cloned at the 5' end encoding the N-terminus, but the amount of the gene that remained past the 3' end was not disclosed and the procedure for subcloning the gene into the expression vector was only briefly summarized. Furthermore, the previous study does not show

the level of expression of the *S. aureus* Pol III, nor the amount of *S. aureus* Pol III-L that is obtained from the induced cells. Since the previously published procedure could not be repeated and the efficiency of the expression vector could not be assessed, another strategy outlined below had to be developed.

5 The isolated Pol III gene was cloned into a vector that utilizes T7 RNA polymerase for transcription as this process generally expresses a large amount of protein. Hence, the *S. aureus* PolC gene was cloned precisely into the start codon at the NdeI site downstream of the T7 promotor in a pET vector. As the PolC gene contains an internal NdeI site, the entire gene could not be amplified and placed it into
10 the NdeI site of a pET vector. Hence, a three step cloning strategy that yielded the desired clone was devised (See Figure 1). These attempts were quite frustrating initially as no products of cloning in standard *E. coli* strains such as DH5alpha, a typical laboratory strain for preparation of DNA, could be obtained. Finally, a cell that was mutated in several genes affecting DNA stability was useful in obtaining the
15 desired products of cloning.

In brief, the cloning strategy required use of another expression vector (called pET1137kDa) in which the 37 kDa subunit of human RFC, the clamp loader of the human replication system, had been cloned into the pET11 vector. The gene encoding the 37kDa subunit contains an internal NsiI site, which was needed for the
20 precise cloning of the isolated PolC gene. This three step strategy is shown in Figure 1. In the first step, an approximately 2.3 kb section of the 5' section of the gene (encoding the N-terminus of Pol III-L) was amplified using the polymerase chain reaction (PCR). Primers were: upstream
5'-GGTGGTAATTGTCTTGCATATGACAGAGC-3' (SEQ. ID. No. 13);
25 downstream 5'-AGCGATTAAGTGGATTGCCGGGTTGTGATG C-3' (SEQ. ID. No. 14). Amplification was performed using 500 ng genomic DNA, 0.5 mM EDTA, 1 µM of each primer, 1mM MgSO₄, 2 units vent DNA polymerase (New England Biolabs) in 100 µl of vent buffer (New England Biolabs). Forty cycles were performed using the following cycling scheme: 94°C, 1 min; 60°C, 1 min.; 72°C, 2.5
30 min. The product was digested with NdeI (underlined in the upstream primer) and NsiI (an internal site in the product) and the approximately 1.8 kb fragment was gel purified. A pET11 vector containing as an insert the 37 kDa subunit of human

replication factor C (pET1137kDa) was digested with NdeI and NsiI and gel purified. The PCR fragment was ligated into the digested pET1137kDa vector and the ligation reaction was transformed into Epicurean coli supercompetent SURE 2 cells (Stratagene) and colonies were screened for the correct chimera (pET11PolC1) by examining minipreps for proper length and correct digestion products using NdeI and NsiI. In the second step, an approximately 2076 bp fragment containing the DNA encoding the C-terminus of Pol III-L holoenzyme was amplified using as primers: upstream 5'-AGCATCACAACCCGGCAATCCACTTAATCG C-3' (SEQ. ID. No. 15); downstream, 5'-GACTACGCCCATGGGCATTAAATAAATACC-3' (SEQ. ID. No. 16). The amplification cycling scheme was as described above except the elongation step at 72°C was for 2 min. The product was digested with BamHI (underlined in the downstream primer) and NsiI (internal to the product) and the approximately 480 bp product was gel purified and ligated into the pET11PolC1 that had been digested with NsiI/BamHI and gel purified (ligated product is pET11PolC2). To complete the expression vector, an approximately 2080 bp PCR product was amplified over the two NsiI sites internal to the gene using the following primers: upstream 5'-GAAGAT GCA TATAAACGTGCA AGACCTAGT C-3' (SEQ. ID. No. 17), downstream 5'-GTCTGACGCACGAATTGTAAAGTAAGATGCATA G-3' (SEQ. ID. No. 18). The amplification cycling scheme was as described above except the 72°C elongation step was 2 min. The PCR product, and the pET11PolC2 vector, were digested with NsiI and gel purified. The ligation mixture was transformed as described above and colonies were screened for the correct chimera (pET11PolC).

To express Pol III-L holoenzyme, the pET11PolC plasmid was transformed into *E. coli* strain BL21(DE3). 24 L of *E. coli* BL21(DE3)pET11PolC were grown in LB media containing 50 µg/ml ampicillin at 37°C to an OD of 0.7 and then the temperature was lowered to 15°C. Cells were then induced for Pol III-L expression upon addition of 1 mM IPTG to produce the T7 RNA polymerase needed to transcribe PolC holoenzyme. This step was followed by further incubation at 15°C for 18 h. Expression of *S. aureus* Pol III-L holoenzyme was so high that it could easily be visualized by Coomassie staining of a SDS polyacrylamide gel of whole cells (Figure 2A). The expressed protein migrated in the SDS polyacrylamide gel in a position expected for a 165 kDa polypeptide. In this procedure, it is important that

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cells are induced at 15°C, as induction at 37°C produces a truncated version of Pol III-L holoenzyme, of approximately 130 kDa.

Cells were collected by centrifugation at 5°C. Cells (12 g wet weight) were stored at -70°C. The following steps were performed at 4°C. Cells were thawed and lysed in cell lysis buffer as described (final volume = 50 ml) and were passed
5 through a French Press (Amico) at a minimum of 20,000 psi. PMSF (2 mM) was added to the lysate as the lysate was collected from the French Press. DNA was removed and the lysate was clarified by centrifugation. The supernatant was dialyzed for 1 h against Buffer A containing 50 mM NaCl. The final conductivity was
10 equivalent to 190 mM NaCl. Supernatant (24 ml, 208 mg) was diluted to 50 ml using Buffer A to bring the conductivity to 96 mM MgCl₂, and then was loaded onto an 8 ml MonoQ column equilibrated in Buffer A containing 50 mM NaCl. The column was eluted with a 160 ml linear gradient of Buffer A from 50 mM NaCl to 500 mM NaCl. Seventy five fractions (1.3 ml each) were collected (see Figure 2B). Aliquots
15 were analyzed for their ability to synthesize DNA, and 20 µl of each fraction was analyzed by Coomassie staining of an SDS polyacrylamide gel. Based on the DNA synthetic capability, and the correct size band in the gel, fractions 56-65 containing Pol III-L holoenzyme were pooled (22 ml, 31 mg). The pooled fractions were dialyzed overnight at 4°C against 50 mM phosphate (pH 7.6), 5 mM DTT, 0.1 mM
20 EDTA, 2 mM PMSF, and 20 % glycerol (P-cell buffer). The dialyzed pool was loaded onto a 4.5 ml phosphocellulose column equilibrated in P-cell buffer, and then eluted with a 25 ml linear gradient of P-cell buffer from 0 M NaCl to 0.5 M NaCl. Fractions of 1 ml were collected and analyzed in a SDS polyacrylamide gel stained with Coomassie Blue (see Figure 2C). Fractions 20-36 contained the majority of the
25 Pol III-large at a purity of greater than 90 % (5 mg).

Example 7 - *S. aureus* Pol III-L is Not Processive on its Own

The Pol III-L holoenzyme purifies from *B. subtilis* as a single subunit
30 without accessory factors (Barnes, et al., "Purification of DNA Polymerase III of Gram-positive Bacteria," Methods in Enzy., 262:35-42 (1995), which is hereby incorporated by reference). Hence, it seemed possible that it may be a Type I

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Example 8 - Cloning and Purification of *S. aureus* Beta Subunit

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was 5'-TTATATGGATCCCTTAGTAAGTTCTGATTGG-3' (SEQ. ID. No. 20); where the BamHI site used for cloning into pET16b (Novagen) is underlined. The NdeI and BamHI sites were used for directional cloning into pET16 (Figure 3). Amplification was performed using 500 ng genomic DNA, 0.5 mM dNTPs, 1 μ M of each primer, 5 1mM MgSO₄, 2 units vent DNA polymerase in 100 μ l of vent buffer. Forty cycles were performed using the following cycling scheme: 94°C, 1 min; 60°C, 1 min.; 72°C, 1 min. 10s. The 1167 bp product was digested with NdeI and BamHI and purified in a 0.7 % agarose gel. The pure digested fragment was ligated into the pET16b vector which had been digested with NdeI and BamHI and gel purified in a 10 0.7% agarose gel. Ligated products were transformed into *E. coli* competent SURE II cells (Stratagene) and colonies were screened for the correct chimera by examining minipreps for proper length and correct digestion products using NdeI and BamHI.

24 L of BL21(DE3)pETbeta cells were grown in LB containing 50 μ g/ml ampicillin at 37 °C to an O.D. of 0.7, and, then, the temperature was lowered to 15 15°C. IPTG was added to a concentration of 2 mM and after a further 18 h at 15°C to induce expression of *S. aureus* beta (Figure 4A). It is interesting to note that the beta subunit, when induced at 37°C, was completely insoluble. However, induction of cells at 15°C provided strong expression of beta and, upon cell lysis, over 50% of the beta was present in the soluble fraction.

20 Cells were harvested by centrifugation (44 g wet weight) and stored at -70°C. The following steps were performed at 4°C. Cells (44 g wet weight) were thawed and resuspended in 45 ml 1X binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris HCl (final pH 7.5)) using a dounce homogenizer. Cells were lysed using a French Pressure cell (Aminco) at 20,000 psi, and then 4.5 ml of 10 % polyamine P 25 (Sigma) was added. Cell debris and DNA was removed by centrifugation at 13,000 rpm for 30 min. at 4°C. The pET16beta vector places a 20 residue leader containing 10 histidine residues at the N-terminus of beta. Hence, upon lysing the cells, the *S. aureus* beta was greatly purified by chromatography on a nickel chelate resin (Figure 4B). The supernatant (890 mg protein) was applied to a 10 ml HiTrap 30 Chelating Separose column (Pharmacia-LKB) equilibrated in binding buffer. The column was washed with binding buffer, then eluted with a 100 ml linear gradient of

60 mM imidazole to 1 M imidazole in binding buffer. Fractions of 1.35 ml were collected. Fractions were analyzed for the presence of beta in an SDS polyacrylamide gel stained with Coomassie Blue. Fractions 28-52, containing most of the beta subunit, were pooled (35 ml, 82 mg). Remaining contaminating protein was removed by chromatography on MonoQ. The *S. aureus* beta becomes insoluble as the ionic strength is lowered, and, thus, the pool of beta was dialyzed overnight against Buffer A containing 400 mM NaCl. The dialyzed pool became slightly turbid indicating it was at its solubility limit at these concentrations of protein and NaCl. The insoluble material was removed by centrifugation (64 mg remaining) and, then, diluted 2-fold with Buffer A to bring the conductivity to 256. The protein was then applied to an 8 ml MonoQ column equilibrated in Buffer A plus 250 mM NaCl and then eluted with a 100 ml linear gradient of Buffer A from 0.25M NaCl to 0.75 M NaCl; fractions of 1.25 ml were collected (Figure 4C). Under these conditions, approximately 27 mg of the beta flowed through the column and the remainder eluted in fractions 1-18 (24 mg).

Example 9 - The *S. aureus* Beta Subunit Protein Stimulates *S. aureus* Pol III-L and *E. coli* Core.

The experiment of Figure 5A, tests the ability of *S. aureus* beta to stimulate *S. aureus* Pol III-L on a linear polydA-oligodT template. Reactions are also performed with *E. coli* beta and Pol III core. The linear template was polydA of average length of 4500 nucleotides primed with a 30mer oligonucleotide of T residues. The first two lanes show the activity of Pol III-L either without (lane 1) or with *S. aureus* beta (lane 2). The result shows that the *S. aureus* beta stimulates Pol III-L approximately 5-6 fold. Lanes 5 and 6 show the corresponding experiment using *E. coli* core with (lane 6) or without (lane 5) *E. coli* beta. The core is stimulated over 10-fold by the beta subunit under the conditions used.

Although gram positive and gram negative cells diverged from one another long ago and components of one polymerase machinery would not be expected to be interchangeable, it was decided to test the activity of the *S. aureus* beta with *E. coli* Pol III core. Lanes 3 and 4 shows that the *S. aureus* beta also stimulates *E. coli* core about 5-fold. This result can be explained by an interaction between the

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clamp and the polymerase that has been conserved during the evolutionary divergence of gram positive and gram negative cells. A chemical inhibitor that would disrupt this interaction would be predicted to have a broad spectrum of antibiotic activity, shutting down replication in gram negative and gram positive cells alike. This assay, and
5 others based on this interaction, can be devised to screen chemicals for such inhibition. Further, since all the proteins in this assay are highly overexpressed through recombinant techniques, sufficient quantities of the protein reagents can be obtained for screening hundreds of thousands of compounds.

In summary, the results show that *S. aureus* beta, produced in *E. coli*,
10 is indeed an active protein (i.e. it stimulates polymerase activity). Furthermore, the results shows that Pol III-L functions with a second protein (i.e. *S. aureus* beta). Before this experiment, there was no assurance that Pol III-L, which is significantly different in structure from *E. coli* alpha, would function with another protein. For example, unlike *E. coli* alpha, which copurifies with several accessory proteins, Pol
15 III-L purified from *B. subtilis* purifies as a single protein with no other subunits attached (Barnes, et al., "Purification of DNA Polymerase III of Gram-positive Bacteria," Methods in Enzy., 262:35-42 (1995), which is hereby incorporated by reference). Finally, if one were to assume that *S. aureus* beta would function with a
20 polymerase, the logical candidate would have been the product of the dnaE gene instead of PolC (Pol III-L) since the dnaE product is more homologous to *E. coli* alpha subunit than Pol III-L.

Example 10 - The *S. aureus* Beta Subunit Behaves as a Circular Sliding Clamp

25 The ability of *S. aureus* beta to stimulate Pol III-L could be explained by formation of a 2-protein complex between Pol III-L and beta to form a processive replicase similar to the Type II class (e.g. T7 type). Alternatively, the *S. aureus* replicase is organized as the Type III replicase which operates with a circular sliding clamp and a clamp loader. In this case, the *S. aureus* beta would be a circular protein
30 and would require a clamp loading apparatus to load it onto DNA. The ability of the beta subunit to stimulate Pol III-L in Figure 5A could be explained by the fact that the polydA-oligodT template is a linear DNA and a circular protein could thread itself

onto the DNA over an end. Such "end threading" has been observed with PCNA and explains its ability to stimulate DNA polymerase delta in the absence of the RFC clamp loader (Burgers. et al., "ATP-Independent Loading of the Proliferating Cell Nuclear Antigen Requires DNA Ends," J. Biol. Chem., 268:19923-19926 (1993),
5 which is hereby incorporated by reference).

To distinguish between these possibilities, *S. aureus* beta was examined for ability to stimulate Pol III-L on a circular primed template. In Figure 5B, assays were performed using circular M13mp18 ssDNA coated with *E. coli* SSB and primed with a single oligonucleotide to test the activity of beta on
10 circular DNA. Lane 1 shows the extent of DNA synthesis using Pol III-L alone. In lane 2, Pol III-L was supplemented with *S. aureus* beta. The *S. aureus* beta did not stimulate the activity of Pol III-L on this circular DNA (nor in the absence of SSB). Inability of *S. aureus* beta to stimulate Pol III-L is supported by the results of Figure 6, lane 1 that analyzes the product of Pol III-L action on the circular DNA in
15 an agarose gel in the presence of *S. aureus* beta. In summary, these results show that *S. aureus* beta only stimulates Pol III-L on linear DNA, not circular DNA. Hence, the *S. aureus* beta subunit behaves as a circular protein.

Lane 3 shows the result of adding both *S. aureus* beta and *E. coli* gamma complex to Pol III-L. Again, no stimulation was observed (compare with lane
20 1). This result indicates that the functional contacts between the clamp and clamp loader were not conserved during evolution of gram positive and gram negative cells.

Controls for these reactions on circular DNA are shown for the *E. coli* system in Lanes 4-6. Addition of only beta to *E. coli* Pol III core did not result in stimulating the polymerase (compare lanes 4 and 5). However, when gamma
25 complex was included with beta and core, a large stimulation of synthesis was observed (lane 6). In summary, stimulation of synthesis is only observed when both beta and gamma complex were present, consistent with inability of the circular beta ring to assemble onto circular DNA by itself.

Example 11 - Pol III-L Functions as a Pol III-Type Replicase with Beta and Gamma Complex to Become Processive

Next, it was determined whether *S. aureus* Pol III-L requires two
5 components (beta and gamma complex) to extend a primer full length around a
circular primed template. In Figure 6, a template circular M13mp18 ssDNA primed
with a single DNA oligonucleotide was used. DNA products were analyzed in a
neutral agarose gel which resolves starting materials (labeled ssDNA in Figure 6)
from completed duplex circles (labelled RFII for replicative form II). The first two
10 lanes show, as demonstrated in other examples, that Pol III-L is incapable of
extending the primer around the circular DNA in the presence of only *S. aureus* beta.
In lane 4 of Figure 6, *E. coli* gamma complex and beta subunit were mixed with *S.*
aureus Pol III-L in the assay containing singly primed M13mp18 ssDNA coated with
SSB. If the beta clamp, assembled on DNA by gamma complex, provides
15 processivity to *S. aureus* Pol III-L, the ssDNA circle should be converted into a fully
duplex circle (RFII) which would be visible in an agarose gel analysis. The results of
the experiment showed that the *E. coli* beta and gamma complex did indeed provide
Pol III-L with ability to fully extend the primer around the circular DNA to form the
RFII (lane 4). The negative control using only *E. coli* gamma complex and beta is
20 shown in lane 3. For comparison, lane 6 shows the result of mixing the three
components of the *E. coli* system (Pol III core, beta and gamma complex). This
reaction gives almost exclusively full length RFII product. The qualitatively different
product profile that Pol III-L gives in the agarose gel analysis compared to *E. coli* Pol
III core with beta and gamma complex shows that the products observed using Pol III-
25 L is not due to a contaminant of *E. coli* Pol III core in the *S. aureus* Pol III-L
preparation (compare lanes 4 and 6).

It is generally thought that the polymerase of one system is specific for
its SSB. However, these reactions are performed on ssDNA coated with the *E. coli*
SSB protein. Hence, the *S. aureus* Pol III-L appears capable of utilizing *E. coli* SSB
30 and the *E. coli* beta. It would appear that the only component that is not
interchangeable between the gram positive and gram negative systems is the gamma
complex.

Thus, the *S. aureus* Pol III-L functions as a Pol III type replicase with the *E. coli* beta clamp assembled onto DNA by gamma complex.

Example 12 - Purification of Two DNA Polymerase III-Type Enzymes From *S. aureus* Cells

The MonoQ resin by Pharmacia has very high resolution which would resolve the three DNA polymerases of *S. aureus*. Hence, *S. aureus* cells were lysed, DNA was removed from the lysate, and the clarified lysate was applied onto a MonoQ column. The details of this procedure are: 300 L of *S. aureus* (strain 4220 (a gift of Dr. Pat Schlievert, University of Minnesota)) was grown in 2X LB media at 37°C to an OD of approximately 1.5 and then were collected by centrifugation. Approximately 2 kg of wet cell paste was obtained and stored at -70°C. 122 g of cell paste was thawed and resuspended in 192 ml of cell lysis buffer followed by passage through a French Press cell (Aminco) at 40,000 psi. The resultant lysate was clarified by high speed centrifugation (1.3 g protein in 120 ml). A 20 ml aliquot of the supernatant was dialyzed 2 h against 2 L of buffer A containing 50 mM NaCl. The dialyzed material (148 mg, conductivity = 101 mM NaCl) was diluted 2-fold with Buffer A containing 50 mM NaCl and then loaded onto an 8 ml MonoQ column equilibrated in Buffer A containing 50 mM NaCl. The column was washed with Buffer A containing 50 mM NaCl, and then eluted with a 160 ml linear gradient of 0.05 M NaCl to 0.5 M NaCl in Buffer A. Fractions of 2.5 ml (64 total) were collected, followed by analysis in an SDS polyacrylamide gel and for their replication activity in assays using calf thymus DNA.

Three peaks of DNA polymerase activity were identified (Figure 7). Previous studies of cell extracts prepared from the gram positive organism, *Bacillus subtilis*, identified only two peaks of activity off a DEAE column (similar charged resin to MonoQ). The first peak was Pol II, and the second peak was a combination of DNA polymerases I and III. The DNA polymerases I and III were then separated on a subsequent phosphocellulose column. The middle peak in Figure 7 is much larger than the other two peaks, and, thus, it was decided to chromatograph this peak on a phosphocellulose column. The second peak of DNA synthetic activity was pooled (fractions 37-43; 28 mg in 14 ml) and dialyzed against 1.5 L P-cell buffer for

2.5 h. Then, the sample (ionic strength equal to 99 mM NaCl) was applied to a 5 ml phosphocellulose column equilibrated in P-cell buffer. After washing the column in 10 ml P-cell buffer, the column was eluted with a 60 ml gradient of 0 - 0.5 M NaCl in P-cell buffer. 70 fractions were collected. Fractions were analyzed for DNA

5 synthesis using calf thymus DNA as template.

This column resolved the polymerase activity into two distinct peaks (Figure 7B). Hence, there appear to be four DNA polymerases in *Staphylococcus aureus*, which was designated here as peaks 1 (first peak off MonoQ), peak 2 (first peak off phosphocellulose), peak 3 (second peak of phosphocellulose), and peak 4 (last peak off Mono Q) (see Figure 7). Peak 4 was presumably Pol III-L, as it elutes from MonoQ in a similar position as the Pol III-L expressed in *E. coli* (compare Figure 7A with Figure 2).

To test which peak contained a Pol III-type of polymerase, an assay was used in which the *E. coli* gamma complex and beta support formation of full length RFII product starting from *E. coli* SSB coated circular M13mp18 ssDNA primed with a single oligonucleotide. In Figure 8, both Peaks 1 and 2 are stimulated by the *E. coli* gamma complex and beta subunit, and, in fact, Peaks 2 and 3 are inhibited by these proteins (the quantitation is shown below the gel in the figure). Further, the product analysis in the agarose gel shows full length RFII duplex DNA circles only for peaks 1 and 4. These results, combined with the NEM, pCMB and KCl characteristics in Table 1 below, suggests that there are two Pol III-type DNA polymerases in *S. aureus*, and that these are partially purified in peaks 1 and 4.

Next, it was determined which of these peaks of DNA polymerase activity correspond to DNA polymerases I, II, and III, and which peak is the unidentified DNA polymerase. In the gram positive bacterium *B. subtilis*, Pol III is inhibited by pCMB, NEM, and 0.15 M NaCl, Pol II is inhibited by KCl, but not NEM or 0.15 M KCL, and Pol I is not inhibited by any of these treatments (Gass, et al., "Further Genetic and Enzymological Characterization of the Three *Bacillus subtilis* Deoxyribonucleic Acid Polymerases," *J. Biol. Chem.*, 248:7688-7700 (1973), which is hereby incorporated by reference). Hence, assays were performed in the presence or absence of pCMB, NEM, and 0.15 M KCl (see Table 1 below). Peak 3 clearly corresponded to Pol I, because it was not inhibited by NEM, pCMB, or 0.15 M NaCl.

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Peak 2 correspond to Pol II, because it was not inhibited by NEM, but was inhibited by pCMB and 0.15 M NaCl. Peaks 1 and 4 both had characteristics that mimic Pol III; however, peak 4 elutes on MonoQ at a similar position as Pol III-L expressed in *E. coli* (see Figure 2B). Hence, peak 4 is likely Pol III-L, and peak 1 is likely the unknown polymerase.

Table 1

Expected Characteristics Polymerase	pCMB	NEM	0.15M KCl
Pol I	not inhibited*	not inhibited	not inhibited
Pol II	inhibited**	not inhibited	not inhibited
Pol III-L	inhibited	inhibited	not inhibited

*Not inhibited is defined as greater than 75% remaining activity

** Inhibited is defined as less than 40% remaining activity

Observed Characteristics			
Peak	PCMB	NEM	0.15M KCL assignment
peak1	inhibited	inhibited	new polymerase
Peak2	inhibited	not inhibited	Pol II
Peak3	not inhibited	not inhibited	Pol I
Peak4	inhibited	inhibited	Pol III-L

Example 13 - Demonstration That Peak 1 (Pol III-2) Functions as a Pol III-Type Replicase With *E. coli* Beta Assembled on DNA by *E. coli* Gamma Complex.

It is interesting to note that the characteristics of peak 1 are similar to those of a Pol III-type of DNA polymerase. To test whether peak 1 contained a Pol III type of polymerase, an assay in which the *E. coli* gamma complex and beta support formation of full length RFII product starting from *E. coli* SSB coated circular M13mp18 ssDNA primed with a single oligonucleotide was carried out. In Figure 8, both Peaks 1 and 2 are stimulated by the *E. coli* gamma complex and beta subunit, and, in fact, Peaks 2 and 3 are inhibited by these proteins (the quantitation is shown below the gel in the Figure 8). Further, the product analysis in the agarose gel shows full length RFII duplex DNA circles only for peaks 1 and 4. These results, combined

with the NEM, pCMB, and KCl characteristics in the Table above, suggests that there are two Pol III-type DNA polymerases in *S. aureus*, and that these are partially purified in peaks 1 and 4.

5 **Example 14 - Identification and Cloning of *S. aureus* dnaE**

 This invention describes the finding of two DNA polymerases that function with a sliding clamp assembled onto DNA by a clamp loader. One of these DNA polymerases is likely Pol III-L, but the other has not been identified previously.

10 Presumably the chromatographic resins used in earlier studies did not have the resolving power to separate the enzyme from other polymerases. This would be compounded by the low activity of Pol III-2. To identify a gene encoding a second Pol III, the amino acid sequences of the Pol III alpha subunit of *Escherichia coli*, *Salmonella typhimurium*, *Vibrio cholerae*, *Haemophilis influenzae*, and *Helicobacter*

15 *pylori* were aligned using Clustal W (1.5). Two regions about 400 residues apart were conserved and primers were designed for the following amino acid sequences: upstream, LLFERFLNPERVSM (SEQ. ID. No. 21) (corresponds in *E. coli* to residues 385-399); downstream KFAGYGFNKSLSAAY (SEQ. ID. No. 22) (corresponds in *E. coli* to residues 750-764). The following primers were designed to

20 these two peptide regions using codon preferences for *S. aureus*: upstream, 5' CTTCTTTTGAAGATTTCTAAATAAAGAACGTTATTCAATGCC 3' (SEQ. ID. No. 23); downstream,

 5' ATAAGCTGCAGCATGACTTTTATTAAAACCATACCTGCAAATTT 3' (SEQ. ID. No. 24). Amplification was performed using 2.5 units of Taq DNA

25 Polymerase (Gibco, BRL), 100 ng *S. aureus* genomic DNA, 1 mM of each of the four dNTPs, 1 μ M of each primer, and 3 mM MgCl₂ in 100 μ l of Taq buffer. Thirty-five cycles of the following scheme were repeated: 94°C, 1 min; 55°C, 1 min; 72°C, 90 sec. The PCR product (approximately 1.1 kb) was electrophoresed in a 0.8 % agarose gel, and purified using a GeneClean III kit (Bio 101). The product was then divided

30 equally into ten separate aliquots, and used as a template for PCR reactions, according to the above protocol, to reamplify the fragment for sequencing. The final PCR product was purified using a Qiagen Quiaquick PCR Purification kit, quantitated via

optical density at 260 nM, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The same primers used for PCR were used to prime the sequencing reactions.

Next, additional PCR primers were designed to obtain more sequence
5 information 3' to the amplified section of the sequence of *dnaE*. The upstream primer was: 5' AGTTAAAAATGCCATATTTTGACGTGTTTGTAGTTCTAAT 3' (SEQ. ID. No. 25), and the downstream primer was,
5' CTTGCAAAAGCGGTTGCTAAAGATGTTGGACGAATTATGGGG 3' (SEQ. ID. No. 26).

10 These primers were used in a PCR reaction using 2.5 units of Taq DNA Polymerase (Gibco, BRL) with 100 ng. *S. aureus* genomic DNA as a template, 1mM dNTP's, 1 M of each primer, 3 mM MgCl₂ in 100 l of Taq buffer. Thirty-five cycles of the following scheme were repeated: 94°C, 1 min; 55°C, 1 min; 72°C, 2 min
15 30 seconds. The 1.6 Kb product was then divided into 5 aliquots, and used as a template in a set of 5 PCR reactions, as described above, to amplify the product for sequencing. The products of these reactions were purified using a Qiagen Qiaquick PCR Purification kit, quantitated via optical density at 260 nm, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The sequence of this
20 product yielded about 740 bp of new sequence 3' of the first sequence. As this gene shows better homology to gram negative pol III subunit compared to gram positive Pol III-L, it will be designated the *dnaE* gene.

As this gene shows better homology to the gram negative Pol III α subunit compared to gram positive Pol III-L, it will be designated the *dnaE* gene.

25 **Example 15 - Identification and Cloning of *S. aureus* *dnaX***

The fact that the *S. aureus* beta stimulates Pol III-L and has a ring
shape suggests that the gram postive replication machinery is of the three component type. This implies the presence of a clamp loader complex. This is not a simple
30 determination to make as the *B. subtilis* genome shows homologs to only two of the five subunits of the *E. coli* clamp loader (*dnaX* encoding gamma, and *holB* encoding delta prime). On the basis of the experiments in this application, which suggests that

there is a clamp loader, we now presume these two subunit homologues are part of the clamp loader for the *S. aureus* beta.

As a start in obtaining the clamp loading apparatus, a strategy was devised to obtain the gene encoding the tau/gamma subunit of *S. aureus*. In *E. coli*,
5 these two subunits are derived from the same gene. Tau is the full length product, and gamma is about 2/3 the length of tau. Gamma is derived from the *dnaX* gene by an efficient translational frameshift mechanism that after it occurs incorporates only one unique C-terminal residue before encountering a stop codon. To identify the *dnaX* gene of *S. aureus* by PCR analysis, the *dnaX* genes of *B. subtilis*, *E. coli*, and *H.*
10 *influenzae* were aligned. Upon comparison of the amino acid sequence encoded by these *dnaX* genes, two areas of high homology were used to predict the amino acid sequence of the *S. aureus* *dnaX* gene product. PCR primers were designed to these sequences, and a PCR product of the expected size was indeed produced. DNA primers were designed to two regions of high similarity for use in PCR that were
15 about 100 residues apart. The amino acid sequences of these regions were: upstream, HAYLFSGPRG (SEQ. ID. No. 27) (corresponds to residues 39-48 of *E. coli*), and downstream, ALLKTLEEPPE (SEQ. ID. No. 28) (corresponds to residues 138-148 of *E. coli*). The DNA sequence of the PCR primers was based upon the codon usage of *S. aureus*. The upstream 38mer was
20 5'-CGCGGATCCCATGCATATTTATTTTCAGGTCCAAGAGG-3' (SEQ. ID. No. 29). The first 9 nucleotides contain a BamHI site and do not correspond to amino acid codons; the 3' 29 nucleotides correspond to the amino acids: HAYLFSGPRG (SEQ. ID. No. 30). The downstream 39 mer was
5'-CCGGAATTCTGGTGGTTCCTTCTAATGTTTTTAATAATGC-3' (SEQ. ID.
25 No. 31). The EcoRI site is underlined and the 3' 33 nucleotides correspond to the amino acid sequence: ALLKTLEEPPE (SEQ. ID. No. 32). The expected PCR product, based on the alignment, is approximately 268 bp between the primer sequences. Amplification was performed using 500 ng genomic DNA, 0.5 mM dNTPs, 1 μ M of each primer, 1 mM MgSO₄, 2 units vent DNA polymerase in 100 μ l
30 of vent buffer. Forty cycles were performed using the following cycling scheme: 94°C, 1 min.; 60°C, 1 min.; 72°C, 30s. The approximately 300 bp product was digested with EcoRI and BamHI and purified in a 0.7 % agarose gel. The pure

5 products using EcoRI and BamHI. The sequence of the insert was determined and was found to have high homology to the dnaX genes of several bacteria. This sequence was used to design circular PCR primers. Two new primers were designed for circular PCR based on this sequence.

10 HincII digest of chromosomal DNA that was recircularized with ligase. This first circular PCR yielded most of the remaining dnaX gene. The rightward directed primer was 5'-TTT GTA AAG GCA TTA CGC AGG GGA CTA ATT CAG ATG TG-3' (SEQ. ID. No. 33); the sequence of the leftward primer was 5'-TAT GAC ATT CAT TAC AAG GTT CTC CAT CAG TGC-3' (SEQ. ID. No. 34).

Genomic DNA (3 μ g) was digested with HincII, purified with phenol/chloroform extraction, ethanol precipitated and redissolved in 70 μ l T.E. buffer. The genomic DNA was recircularized upon adding 4000 units T4 ligase (New England Biolabs) in a final volume of 100 μ l T4 ligase buffer (New England Biolabs) at 16°C overnight. The PCR reaction consisted of 90 ng recircularized genomic DNA, 0.5 mM each dNTP, 100 pmol of each primer, 1.4 mM magnesium sulfate, and 1 unit of elongase (GIBCO) in a final volume of 100 μ l elongase buffer (GIBCO). 40 cycles were performed using the following scheme: 94°C, 1 min., 55°C, 1 min., and 68°C, 2 min. The resulting PCR product was approximately 1.6 kb. The PCR product was purified from a 0.7 % agarose gel and sequenced directly. A stretch of approximately 750 nucleotides was obtained using the rightward primer used in the circular PCR reaction. To obtain the rest of the sequence, other sequencing primers were designed in succession based on the information of each new sequencing run.

present upstream) and possibly lacked only about 50 residues of the C-terminus. The amino terminal region of *E. coli* gamma/tau shares appears the most conserved region of the gene as this area shares homology with RFC subunit of the human clamp loader

and with the gene 44 protein of the phage T4 clamp loader. An alignment of the N-terminal region of the *S. aureus* gamma/tau protein with that of *B. subtilis* and *E. coli* is shown in Figure 10. Among the highly conserved residues are the ATP binding site consensus sequence and the four cystine residues that form a Zn⁺⁺ finger.

5 After obtaining 1 kb of sequence in the 5' region of dnaX, it was sought to determine the remaining 3' end of the gene. Circular PCR products of approximately 800bps, 600bps, and 1600bps were obtained from Apo I, or Nsi I or Ssp I digest of chromosomal DNA that were recircularized with ligase. The rightward directed primer was 5'-GAGCACTGATGAACTTAGAATTAGATATG-3' (SEQ. ID. No. 35); the sequence of the leftward primer was 5'-
10 GATACTCAGTATCTTTCTCAGATGTTTTATTC-3' (SEQ. ID. No. 36). Genomic DNA (3 g) was digested with, Apo I, or Nsi I or Ssp I, purified with phenol/chloroform extraction, ethanol precipitated, and redissolved in 70 l T.E. buffer. The genomic DNA was recircularized upon adding 4000 units of T4 ligase
15 (New England Biolabs) in a final volume of 100 l T4 ligase buffer (New England Biolabs) at 16°C overnight. The PCR reaction consisted of 90 ng recircularized genomic DNA, 0.5 mM each dNTP, 100 pmol of each primer, 1.4 mM magnesium sulfate, and 1 unit of elongase (GIBCO) in a final volume of 100 l elongase buffer (GIBCO). 40 cycles were performed using the following scheme: 94°C, 1 min.;
20 55°C, 1 min.; 68°C, 2 min. The PCR products were directly cloned into pCR II TOPO vector using the TOPO TA cloning kit (Invitrogen Corporation) for obtaining the rest of the C terminal vector using the TOPO TA cloning kit (Invitrogen Corporation) for obtaining the rest of the C terminal sequence of *S. aureus* dnaX. DNA sequencing was performed by the Rockefeller University sequencing facility.

25

Example 16 - Identification and Cloning of *S. aureus* dnaB

 In *E. coli*, the DnaB helicase assembles with the DNA polymerase III holoenzyme to form a replisome assembly. The DnaB helicase also interacts directly
30 with the primase to complete the machinery needed to duplicate a double helix. As a first step in studying how the *S. aureus* helicase acts with the replicase and primase, *S. aureus* was examined for presence of a dnaB gene.

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The amino acid sequences of the DnaB helicase of *Escherichia coli*, *Salmonella typhimurium*, *Haemophilis influenzae*, and *Helicobacter pylori* were aligned using Clustal W (1.5). Two regions about 200 residues apart showed good homology. These peptide sequences were: upstream, DLIIVAARPSMGKT (SEQ. ID. No. 37) (corresponds to residues 225-238 of *E. coli* DnaB), and downstream, EIIIGKQRNGPIGTV (SEQ. ID. No. 38) (corresponds to residues 435-449 of *E. coli*). The following primers were designed from regions which contained conserved sequences using codon preferences for *S. aureus*: The upstream primer was 5' GACCTTATAATTGTAGCTGCACGTCC TTCTAT GGGAAAAAC 3' (SEQ. ID. No. 39); the downstream primer was 5' AACATTATTAAGTCAGCATCTTGT TCTATTGATCCAGATTCAACGAAG 3' (SEQ. ID. No. 40). A PCR reaction was carried out using 2.5 units of Taq DNA Polymerase (Gibco, BRL) with 100 ng. *S. aureus* genomic DNA as template, 1 mM dNTP's, 1 μ M of each primer, 3 mM MgCl₂ in 100 μ l of Taq buffer. Thirty-five cycles of the following scheme were repeated: 94°C, 1 min.; 55°C, 1 min.; and 72°C, 1 min. Two PCR products were produced, one was about 1.1 kb, and another was 0.6 kb. The smaller one was the size expected. The 0.6 kb product was gel purified and used as a template for a second round of PCR as follows. The 0.6 kb PCR product was purified from a 0.8% agarose gel using a GeneClean III kit (Bio 101) and then divided equally into five separate aliquots, as a template for PCR reactions. The final PCR product was purified using a Quiagen Quiaquick PCR Purification kit, quantitated via optical density at 260 nM, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The same primers used for PCR were used to prime the sequencing reaction. The amino acid sequence was determined by translation of the DNA sequence in all three reading frames, and selecting the longest open reading frame. The PCR product contained an open reading frame over its entire length. The predicted amino acid sequence shares homology to the amino acid sequences encoded by dnaB gene of other organisms.

Additional sequence information was determined using the circular PCR technique. Briefly, *S. aureus* genomic DNA was digested with various endonucleases, then religated with T4 DNA ligase to form circular templates. To perform PCR, two primers were designed from the initial sequence. The first primer,

5' GATTTGTAGTTCTGGTAATGTTGACTCAAACCGCTTAAGAACCGG 3'
(SEQ ID. No. 41), matches the coding strand; the second primer, 5'
ATACGTGTGGTTAACTGATCAGCAACCCATCTCTAGTGAGAAAATACC 3'
(SEQ ID. No. 42), matches the sequence of the complementary strand. These two
5 primers are directed outwards from a central point, and allow determination of new
sequence information up to the ligated endonuclease site. A PCR product of
approximately 900 bases in length was produced using the above primers and
template derived from the ligation of *S. aureus* genomic DNA which had been cut
with the restriction endonuclease Apo I. This PCR product was electrophoresed in a
10 0.8% agarose gel, eluted with a Qiagen gel elution kit, divided into five separate
aliquots, and used as a template for reamplification by PCR using the same primers as
described above. The final product was electrophoresed in an 0.8% agarose gel,
visualized via staining with ethidium bromide under ultraviolet light, and excised
from the gel. The excised gel slice was frozen, and centrifuged at 12,000 rpm for 15
15 minutes. The supernatant was extracted with phenol/chloroform to remove ethidium
bromide, and was then cleaned using a Qiagen PCR purification kit. The material was
then quantitated from its optical density at 260 nm and sequenced by the Protein/DNA
Technology Center at the Rockefeller University.

The nucleotide sequence contained an open reading frame over its
20 length, up to a sequence which corresponded to the consensus sequence of a cleavage
site of the enzyme Apo I. Following this point, a second open reading frame encoded
a different reading frame up to the end of the product. The initial sequence
information was found to match the initial sequence and to extend it yet further
towards the C-terminus of the protein. The second reading frame was found to end in
25 a sequence which matched the 5'-terminus of the previously determined sequence and,
thus, represents an extension of the sequence towards the N-terminus of the protein.

Additional sequence information was obtained using the above primers
and a template generated using *S. aureus* genomic DNA circularized via ligation with
T4 ligase following digestion with Cla I. The PCR product was generated using 35
30 cycles of the following program: denaturation at 94°C for 1 min.; annealing at 55°C
for 1 min.; and extension at 68°C for 3 minutes and 30 s. The PCR products were
electrophoresed in a 0.8% agarose gel, eluted with a Qiagen gel elution kit, divided

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into five separate aliquots, and used as a template reamplification via PCR with the same primers described above. The final product was electrophoresed in an 0.8% agarose gel, visualized via staining with ethidium bromide under ultraviolet light, and excised from the gel. The excised gel slice was frozen, and centrifuged at 12,000 rpm
5 for 15 min. The supernatant was cleaned using a Qiagen PCR purification kit. The material was then quantitated via optical density at 260 nm and sequenced by the Protein/DNA Technology Center at Rockefeller University. The open reading frames continued past 500 bases. Therefore, the following additional sequencing primers were designed from the sequence to obtain further information:

10 5' CGTTTAAATGCATGCTTAGAAACGATATCAG 3' (SEQ. ID No. 43) and,
5' CATTGCTAAGCAACGTTACGGTCCAACAGGC 3' (SEQ. ID No. 44).

The N-terminal and C-terminal nucleotide sequence extensions generated using this circular PCR product completed the 5' region of the gene (encoding the N-terminus of DnaB); however, a stop codon was not reached in the 3'
15 region and, thus, a small amount of sequence is still needed to complete this gene.

The alignment of the *S. aureus* dnaB with *E. coli* dnaB and the dnaB genes of *B. subtilis* and *S. typhimurium* is shown in Figure 11.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations
20 can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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WHAT IS CLAIMED:

1. An isolated DNA molecule corresponding to dnaE from a Gram positive bacterium.
5
2. An isolated DNA molecule according to claim 1, wherein the Gram positive bacterium is a *Staphylococcus*.
3. An isolated DNA molecule according to claim 2, wherein the
10 Gram positive bacterium is *Staphylococcus aureus*.
4. An isolated DNA molecule according to claim 3, wherein said DNA molecule encodes a protein having an amino acid sequence of SEQ. ID. No. 2.
- 15 5. An isolated DNA molecule according to claim 3, wherein said DNA molecule has a nucleotide sequence of SEQ. ID. No. 1 or hybridizes under stringent conditions to a nucleotide sequence of SEQ. ID. No. 1.
6. An expression system containing the DNA molecule according
20 to claim 1.
7. An expression system according to claim 6, wherein the DNA molecule is in proper sense orientation and correct reading frame.
- 25 8. A host cell transformed with the DNA molecule according to claim 1.
9. A host cell according to claim 8, wherein the DNA molecule is in an expression system.
30
10. An isolated dnaE protein from a Gram positive bacterium.

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11. An isolated protein according to claim 10, wherein the Gram positive bacterium is a *Staphylococcus*.

12. An isolated protein according to claim 11, wherein the Gram positive bacterium is *Staphylococcus aureus*.

13. An isolated protein according to claim 12, wherein said protein has an amino acid sequence of SEQ. ID. No. 2.

14. An isolated DNA molecule corresponding to dnaX from a Gram positive bacterium.

15. An isolated DNA molecule according to claim 14, wherein the Gram positive bacterium is a *Staphylococcus*.

16. An isolated DNA molecule according to claim 15, wherein the Gram positive bacterium is *Staphylococcus aureus*.

17. An isolated DNA molecule according to claim 16, wherein said DNA molecule encodes a protein having an amino acid sequence of SEQ. ID. No. 4.

18. An isolated DNA molecule according to claim 16, wherein said DNA molecule has a nucleotide sequence of SEQ. ID. No. 3 or hybridizes under stringent conditions to a nucleotide sequence of SEQ. ID. No. 3.

19. An expression system containing the DNA molecule according to claim 16.

20. An expression system according to claim 19, wherein the DNA molecule is in proper sense orientation and correct reading frame.

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21. A host cell transformed with the DNA molecule according to claim 16.
22. A host cell according to claim 21, wherein the DNA molecule
5 is in an expression system.
23. An isolated dnaX protein from a Gram positive bacterium.
24. An isolated protein according to claim 23, wherein the Gram
10 positive bacterium is a *Staphylococcus*.
25. An isolated protein according to claim 24, wherein the Gram positive bacterium is *Staphylococcus aureus*.
- 15 26. An isolated protein according to claim 25, wherein said protein has an amino acid sequence of SEQ. ID. No. 4.
27. An isolated DNA molecule corresponding to dnaB from a Gram positive bacterium.
- 20 28. An isolated DNA molecule according to claim 27, wherein the Gram positive bacterium is a *Staphylococcus*.
29. An isolated DNA molecule according to claim 28, wherein the
25 Gram positive bacterium is *Staphylococcus aureus*.
30. An isolated DNA molecule according to claim 29, wherein said DNA molecule encodes a protein having an amino acid sequence of SEQ. ID. No. 6.
- 30 31. An isolated DNA molecule according to claim 29, wherein said DNA molecule has a nucleotide sequence of SEQ. ID. No. 5 or hybridizes under stringent conditions to a nucleotide sequence of SEQ. ID. No. 5.

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32. An expression system containing the DNA molecule according to claim 27.
- 5 33. An expression system according to claim 32, wherein the DNA molecule is in proper sense orientation and correct reading frame.
34. A host cell transformed with the DNA molecule according to claim 27.
- 10 35. A host cell according to claim 34, wherein the DNA molecule is in an expression system.
36. An isolated dnaB protein from a Gram positive bacterium.
- 15 37. An isolated protein according to claim 36, wherein the Gram positive bacterium is a *Staphylococcus*.
38. An isolated protein according to claim 37, wherein the Gram positive bacterium is *Staphylococcus aureus*.
- 20 39. An isolated protein according to claim 38, wherein the protein has an amino acid sequence of SEQ. ID. No. 6.
- 25 40. A method of identifying compounds which inhibit the activity of a Pol III 2 DNA polymerase or a Pol III L DNA polymerase comprising:
forming a reaction mixture comprising a primed DNA molecule, a DNA polymerase from a Gram positive bacterium, a candidate compound, and a dNTP;
30 subjecting the reaction mixture to conditions effective to achieve nucleic acid polymerization in the absence of the candidate compound;

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analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and

identifying candidate compounds in reaction mixtures where there is an absence of nucleic acid polymerization extension products.

5

41. A method of identifying compounds which inhibit the ability of a beta subunit to stimulate a Pol III 2 DNA polymerase or a Pol III L DNA polymerase comprising:

forming a reaction mixture comprising a primed DNA molecule, a DNA polymerase, a candidate compound, a beta subunit, and a dNTP; subjecting the reaction mixture to conditions effective to achieve nucleic acid polymerization in the absence of the candidate compound, wherein either or both the beta subunit and/or the DNA polymerase are derived from a Gram positive bacterium;

analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and

identifying candidate compounds in reaction mixtures where there is an absence of nucleic acid polymerization extension products.

42. A method of identifying compounds which inhibit the ability of a beta subunit and a gamma complex to stimulate a Pol III 2 DNA polymerase or a Pol III L DNA polymerase comprising:

forming a reaction mixture comprising a primed DNA molecule, a DNA polymerase, a candidate compound, a beta subunit, a gamma complex, and a dNTP;

subjecting the reaction mixture to conditions effective to achieve nucleic acid polymerization in the absence of the candidate compound, wherein either or both of the beta subunit and/or the gamma complex or a subunit or combination of subunits thereof are derived from a Gram positive bacterium;

analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and

identifying candidate compounds in reaction mixtures where there is an absence of nucleic acid polymerization extension products.

43. A method of identifying compounds which inhibit the ability of
5 a beta subunit and a DNA polymerase to interact physically comprising:
forming a reaction mixture comprising a beta subunit, a DNA
polymerase, and a candidate compound;
subjecting the reaction mixture to conditions effective to permit
the beta subunit and the DNA polymerase to interact in the absence of the candidate
10 compound, wherein either or both the beta subunit and/or the DNA polymerase are
derived from a Gram positive bacterium;
analyzing the reaction mixture for the presence or absence of
interaction between the beta subunit and the DNA polymerase; and
identifying candidate compound in reaction mixtures where
15 there is an absence of interaction between the beta subunit and the DNA polymerase.

44. A method of identifying compounds which inhibit the ability of
a beta subunit and a gamma complex or subunit(s) thereof to interact comprising:
forming a reaction mixture comprising a beta subunit, a gamma
20 complex or subunit(s) thereof, and a candidate compound, wherein either or both of
the beta subunit and/or the gamma complex or subunit(s) thereof are derived from a
Gram positive bacterium;
subjecting the reaction mixture to conditions effective to permit
the beta subunit and the gamma complex or subunit(s) thereof to interact in the
25 absence of the candidate compound;
analyzing the reaction mixture for the presence or absence of
interaction between the beta subunit and the gamma complex or subunit(s) thereof;
and
identifying the candidate compound in reaction mixtures where
30 there is an absence of interaction between the beta subunit and the gamma complex or
subunit(s) thereof.

45. A method of identifying compounds which inhibit the ability of a gamma complex or subunit(s) thereof to assemble a beta subunit on a DNA molecule comprising:

- forming a reaction mixture comprising a circular primed DNA molecule, a beta subunit, a gamma complex or subunit(s) thereof, an ATP, and a candidate compound, wherein the beta subunit and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium;
- subjecting the reaction mixture to conditions effective to permit the gamma complex or subunit(s) thereof to assemble the beta subunit on the DNA molecule in the absence of the candidate compound;
- analyzing the reaction mixture for the presence or absence of the beta subunit on the DNA molecule; and
- identifying the candidate compound in reaction mixtures where there is an absence of the beta subunit on the DNA molecule.

15

46. A method of identifying compounds which inhibit the ability of a gamma complex or subunit(s) thereof to disassemble a beta subunit from a DNA molecule comprising:

- forming a reaction mixture comprising a DNA molecule on to which a beta subunit has been assembled, a gamma complex or subunit(s) thereof, and a candidate compound, wherein the beta subunit and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium;
- subjecting the reaction mixture to conditions effective to permit the gamma complex or subunit(s) thereof to disassemble the beta subunit from the DNA molecule in the absence of the candidate compound;
- analyzing the reaction mixture for the presence or absence of the beta subunit on the DNA molecule; and
- identifying the candidate compound in reaction mixtures where the beta subunit is on the DNA molecule.

30

47. A method of identifying compounds which disassemble a beta subunit from a DNA molecule comprising:

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forming a reaction mixture comprising a circular DNA molecule on to which a beta subunit has been assembled, a gamma complex or subunit(s) thereof, and a candidate compound;

5 subjecting the reaction mixture to conditions effective to permit the gamma complex or subunit(s) thereof to disassemble the beta subunit from the DNA molecule in the absence of the candidate compound, wherein either or both the beta subunit and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium;

10 analyzing the reaction mixture for the presence or absence of the beta subunit on the DNA molecule; and

identifying the candidate compound in reaction mixtures where the beta subunit is absent from the DNA molecule.

48. A method of identifying compounds which inhibit dATP/ATP binding activity of a gamma complex or subunit(s) thereof comprising:

15 forming a reaction mixture comprising a gamma complex or subunit(s) thereof, dATP/ATP in the presence or absence of a DNA molecule and/or a beta subunit, and a candidate compound, wherein either or both the beta subunit and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium;

20 subjecting the reaction mixture to conditions effective to permit the gamma complex or subunit(s) thereof to interact with the dATP/ATP in the absence of the candidate compound;

25 analyzing the reaction mixture to determine whether or not the dATP/ATP is bound to the gamma complex or subunit(s) thereof; and

identifying the candidate compound in reaction mixtures where the dATP/ATP is not bound to the gamma complex or subunit(s) thereof.

49. A method of identifying compounds which inhibit dATP/ATP binding activity of a gamma complex or subunit(s) thereof comprising:

30 forming a reaction mixture comprising a gamma complex or subunit(s) thereof, dATP/ATP in the presence or absence of a DNA molecule and/or a

beta subunit, and a candidate compound, wherein either or both the gamma complex or subunit(s) thereof and/or the beta subunit are derived from a Gram positive bacterium:

5 the gamma complex or subunit(s) thereof to hydrolyze dATP/ATP in the absence of
the candidate compound, wherein either or both the gamma complex or subunit(s)
thereof and/or the beta subunit are derived from a Gram positive bacterium;

analyzing the reaction mixture to determine whether or not
dATP/ATP is hydrolyzed; and
10 identifying the candidate compound in reaction mixtures where
dATP/ATP is not hydrolyzed.

50. A method of identifying compounds which inhibit a DNA polymerase encoded by a *dnaE* gene or *PolC* gene comprising:

15 forming a reaction mixture comprising a primed DNA molecule, a protein encoded by a *dnaE* gene or *PolC* gene from a Gram positive bacterium, dNTP or modified dNTP, and a candidate compound;

subjecting the reaction mixture to conditions effective to permit nucleic acid polymerization and the resulting formation of an extension product in the

20 absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of extension product; and

identifying the candidate compound in reaction mixtures where there is an absence of an extension product.

25

51. A method of identifying compounds which inhibit a DNA polymerase encoded by a *dnaE* gene or PolC gene comprising:

forming a reaction mixture comprising a primed, linear DNA molecule, a protein encoded by a *dnaE* gene or PolC gene, dNTP or modified dNTP, a

30 beta subunit, and a candidate compound, wherein either or both the protein encoded by the *dnaE* gene or PolC gene and/or the beta subunit are derived from a Gram positive bacterium;

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subjecting the reaction mixture to conditions effective to permit nucleic acid polymerization and the resulting formation of an extension product in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of
5 extension product; and

identifying the candidate compound in reaction mixtures where there is an absence of an extension product.

52. A method of identifying compounds which inhibit a DNA
10 polymerase encoded by a dnaE gene or PolC gene comprising:
forming a reaction mixture comprising a primed, circular DNA molecule, a protein encoded by a dnaE gene or PolC gene, dNTP or modified dNTP, a beta subunit, a gamma complex or subunit thereof, and a candidate compound,
wherein either or all of the protein encoded by the dnaE gene or PolC gene, the beta
15 subunit, and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium;

subjecting the reaction mixture to conditions effective to permit nucleic acid polymerization and the resulting formation of an extension product in the absence of the candidate compound;

20 analyzing the reaction mixture for the presence or absence of extension product; and

identifying the candidate compound in reaction mixtures where there is an absence of an extension product.

25 53. A method of identifying compounds which inhibit a DNA polymerase encoded by a dnaE gene or PolC gene comprising:

forming a reaction mixture comprising a protein encoded by a dnaE gene or PolC gene, a beta subunit, and a candidate compound, wherein either or both the protein encoded by the dnaE gene or PolC gene and/or the beta subunit are
30 derived from a Gram positive bacterium;

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subjecting the reaction mixture to conditions effective to permit the beta subunit to interact with the protein encoded by the dnaE gene or PolC gene in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of
5 interaction between the beta subunit and the protein encoded by the dnaE gene or PolC gene; and

identifying the candidate compound in reaction mixtures where there is an absence of interaction between the beta subunit and the protein encoded by the dnaE gene or PolC gene.

10

54. A method of identifying compounds which inhibit a DnaB helicase comprising:

forming a reaction mixture comprising a DnaB helicase from a Gram positive bacterium, a substrate DNA molecule having a duplex region, and a
15 nucleoside or deoxynucleoside triphosphate energy source;

subjecting the reaction mixture to conditions effective to support helicase activity in the absence of the candidate compound;

analyzing the reaction mixture for conversion of the duplex DNA molecule to a single stranded DNA molecule; and

20 identifying the candidate compound in reaction mixtures where the duplex DNA molecule is not converted to a single stranded DNA molecule.

55. A method of identifying compounds which inhibit nucleoside or deoxynucleoside triphosphate activity of a DnaB helicase from a Gram positive
25 bacterium comprising:

forming a reaction mixture comprising a DnaB helicase, a substrate DNA molecule having a duplex region, a nucleoside or deoxynucleoside triphosphate energy source, and a candidate compound;

30 subjecting the reaction mixture to conditions effective to support nucleoside or deoxynucleoside activity of DnaB in the absence of the candidate compound;

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analyzing the reaction mixture for conversion of the nucleoside or deoxynucleoside triphosphate to a nucleoside or deoxynucleoside diphosphate; and
identifying the candidate compound in reaction mixtures where the nucleoside or deoxynucleoside triphosphate is not converted to the nucleoside or
5 deoxynucleoside diphosphate.

56. A method of identifying compounds which inhibit primase activity comprising:
forming a reaction mixture comprising a primase from a Gram
10 positive bacterium, a single stranded DNA molecule, and a candidate compound;
subjecting the reaction mixture to conditions effective to support primase activity in the absence of the candidate compound;
analyzing the reaction mixture for the presence or absence of primer formation; and
15 identifying the candidate compound in reaction mixtures where no primers are formed.

57. A method of identifying compounds which inhibit the ability of a primase and a DnaB protein to interact comprising:
20 forming a reaction mixture comprising a primase, a DnaB protein, and a candidate compound, wherein either or both the primase and/or DnaB are derived from a Gram positive bacterium;
subjecting the reaction mixture to conditions effective to permit the primase and the DnaB protein to interact in the absence of the candidate
25 compound;
analyzing the reaction mixture for the presence or absence of interaction between the primase and the DnaB protein; and
identifying the candidate compound in reaction mixtures where no interaction occurs between the primase and the DnaB protein.

30

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58. A method of identifying compounds which inhibit the ability of a DNA molecule and a DnaB protein from a Gram positive bacterium to interact comprising:

- forming a reaction mixture comprising a DNA molecule, a
- 5 DnaB protein from a Gram positive bacterium, and a candidate compound;
- subjecting the reaction mixture to conditions effective to permit the DNA molecule and the DnaB protein to interact in the absence of the candidate compound;
- analyzing the reaction mixture for the presence or absence of
- 10 interaction between the DNA molecule and the DnaB protein; and
- identifying the candidate compound in reaction mixtures where no interaction occurs between the DNA molecule and the DnaB protein.

59. A method according to any one of claims 40 to 58, wherein the

15 Gram positive bacterium is a *Staphylococcus*.

60. A method according to any one of claims 40 to 58, wherein the Gram positive bacterium is a *Staphylococcus aureus*.

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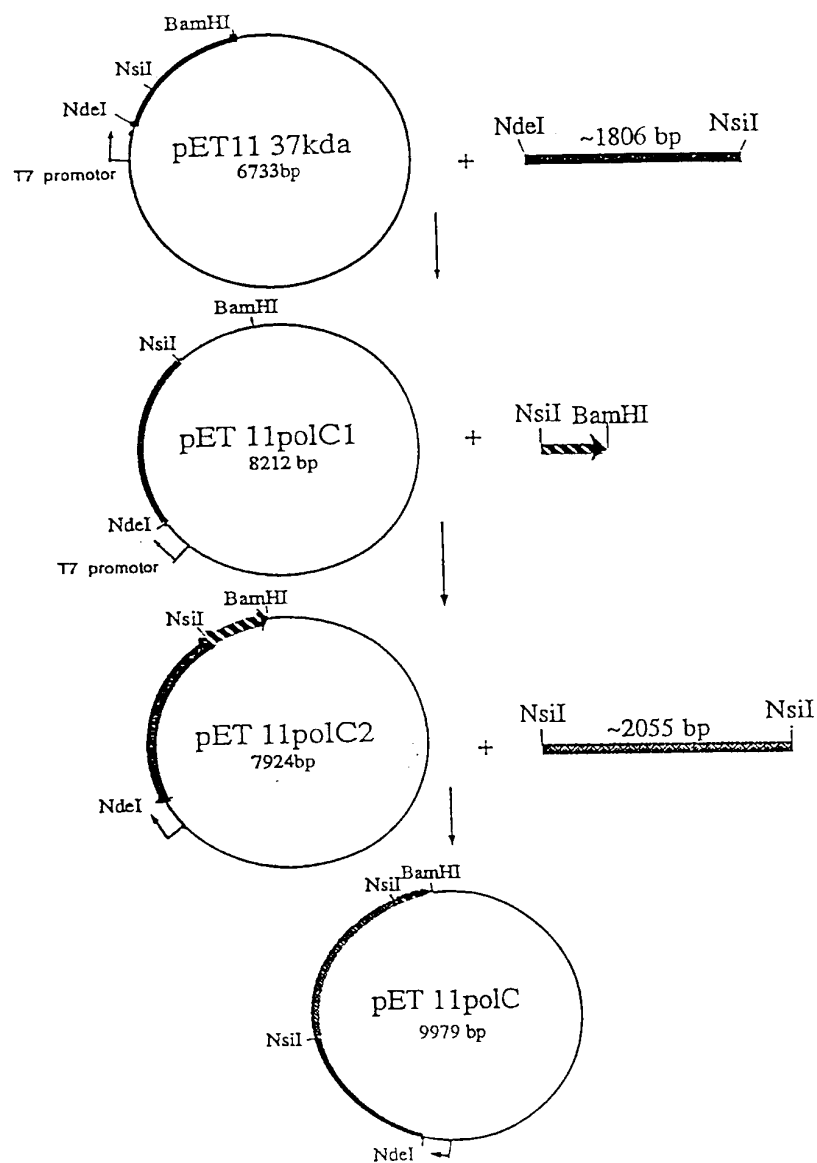


FIGURE 1

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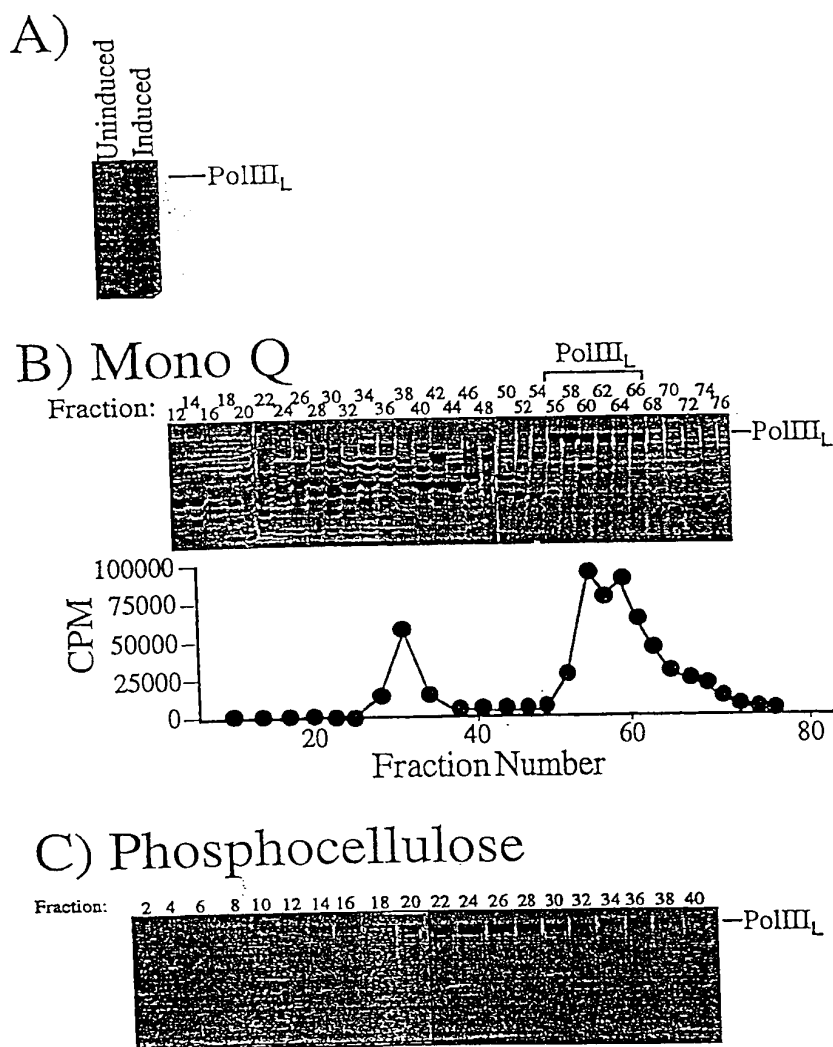


FIGURE 2

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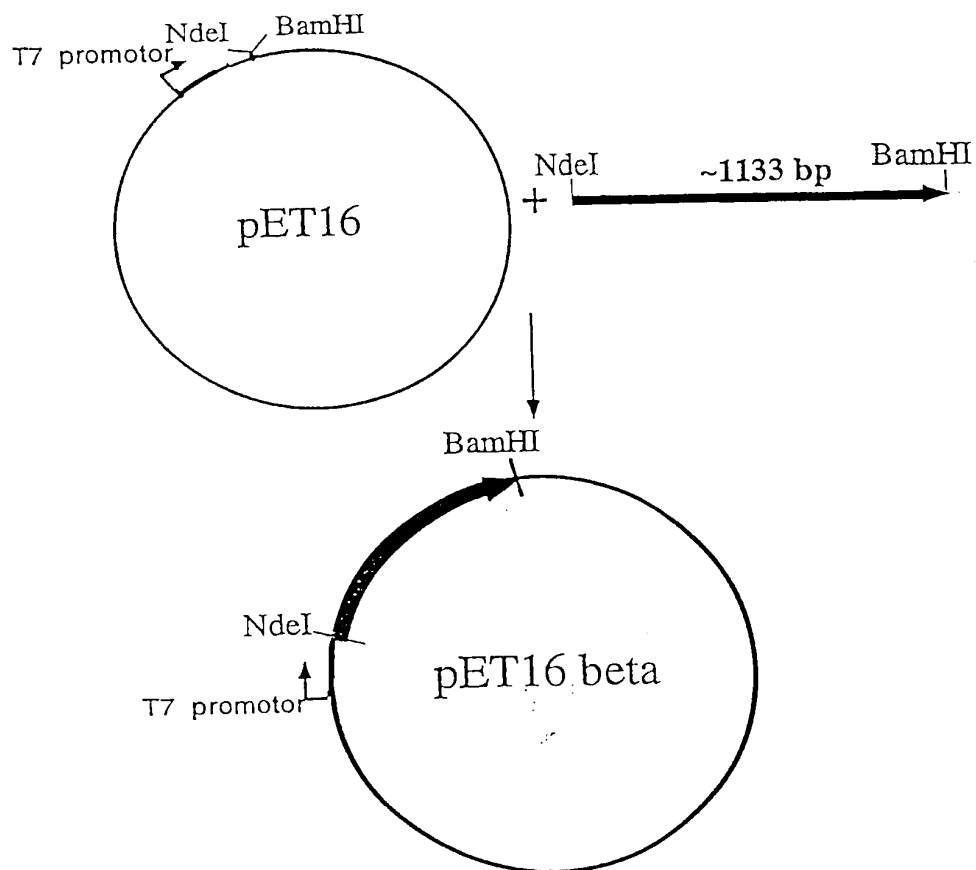


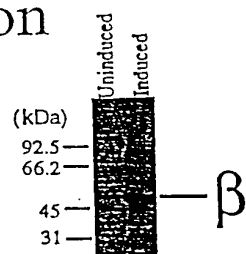
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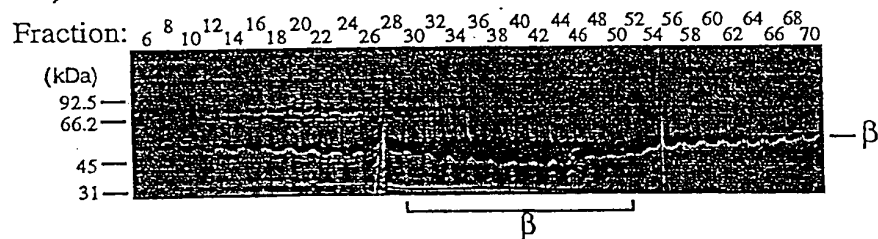
PCT/US99/01547

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A) Induction



B) Nickel column



C) Mono Q

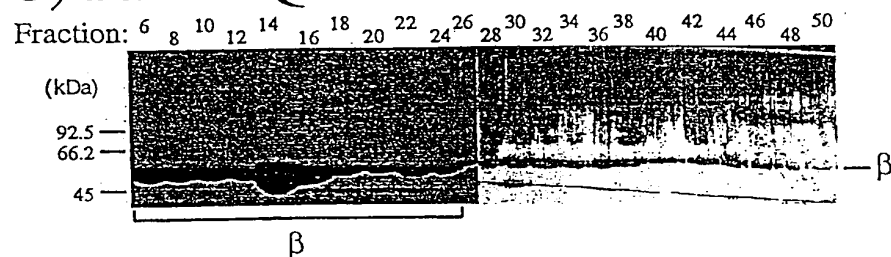


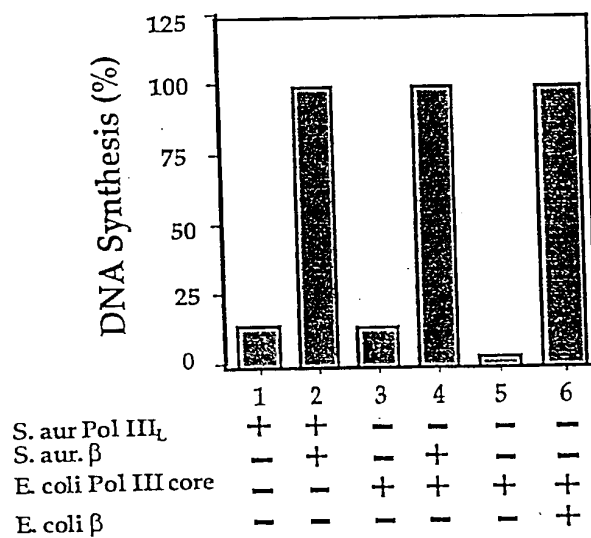
FIGURE 4

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A) Linear DNA



B) Circular DNA

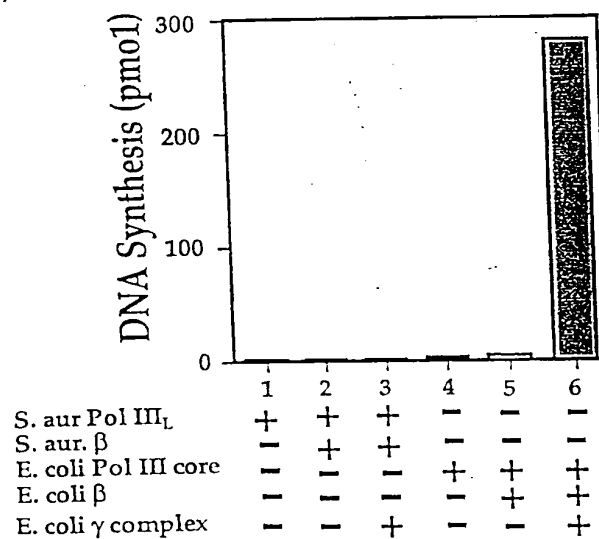


FIGURE 5

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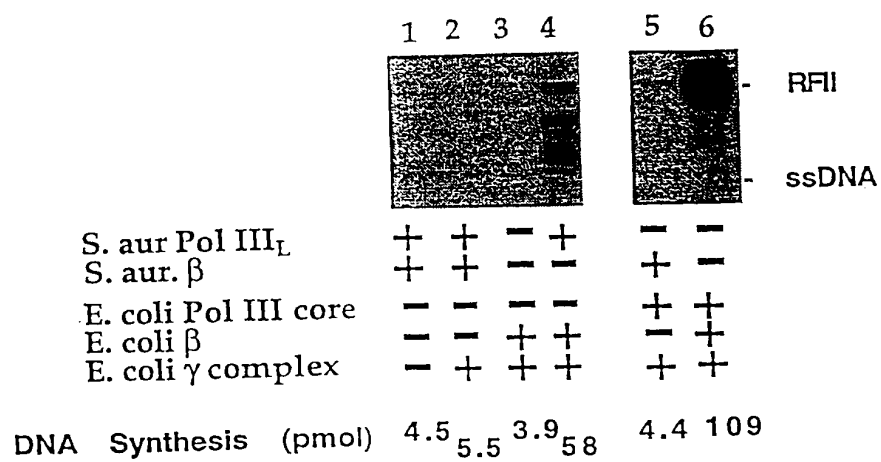


FIGURE 6

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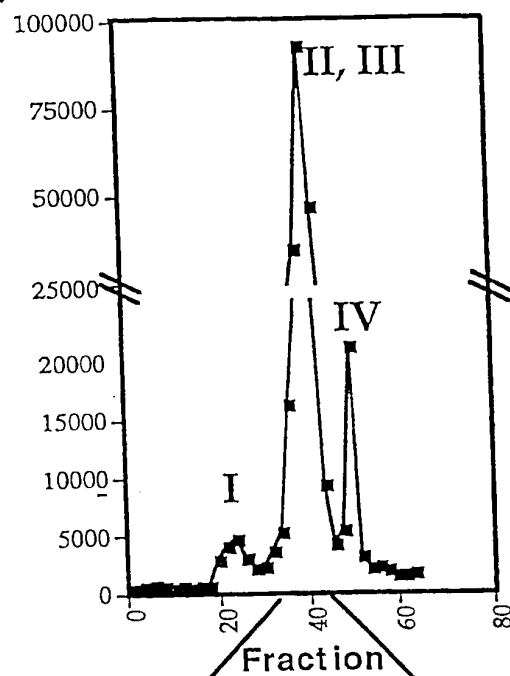
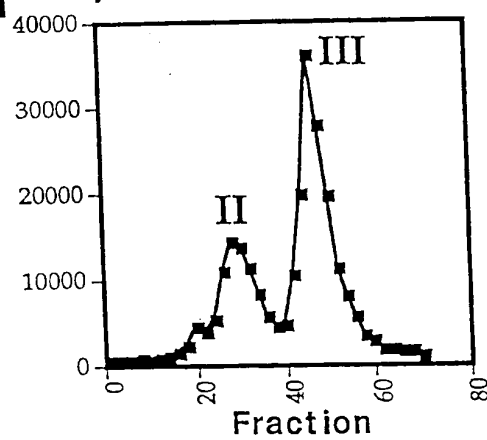
A) MonoQ**B) P-Cell**

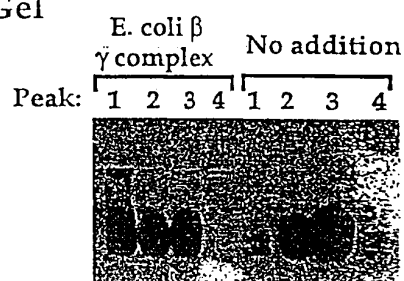
FIGURE 7

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A) Agarose Gel



B) DNA Synthesis

Addition	DNA Synthesis (pmol)			
	PEAK			
	Peak 1	Peak 2	Peak 3	Peak 4
None	22.7	70.6	146.1	4.7
E. coli β , γ complex	72.9	61.2	71.4	25.9

FIGURE 8

S. aureus
E. coli
Sal. typ

S. aureus
E. coli
Sal. typ

S. aureus
E. coli
Sal. typ

S. aureus
E. coli
Sal. typ

S. aureus
E. coli
Sal. typ

-----KQYLHPHLEPIKNTYGVILLYOEQLMQASIFANFSIGSDILKAKKPEEK
ISYPDVQMOHESLKPVLEPTGYIILYQEOVMQIAVLSGYTLGGADMLRRAMGKKRPEEK
LSYPDVQMOHESLKPVLEPTGYIILYQEOVMQIAVLSGYTLGGADMLRRAMGKKRPEEK

S. aureus
E. coli
Sal. typ

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ATP site

S. aureus#KGYCLWRCNLIDYQALFVPTP-KPEDVVGQEHSEDCAMG-----SHAYLFSGPRGTGKT
B. sub. -----MSYQALYRVFRPQRFEDVVGQEHITKTQNALLOKKESHAYLFSGPRGTGKT
E. coli -----MSYQVILARKWRPQTFADVVGQEHVLTALANGLSLGRTHAYLFSGTRGVGKT
*** * * * *
*** * * * *

Zn++ finger

S. aureusSIKVFKA INCLNSTDGEPCNECHICKGITQGTNSDVIEIDAASNGVDEIRNIRDKVKYA
B. sub SAKIFAKAVNCEHAPVDEPCNECAACKGITNGSISDVIEIDAASNGVDEIRDKVKA
E. coli SIARLLAKGLNCEGTITATPCGVCDCNCREIEQGRFVDLIEIDAASRTKVEDTRDLLDNVQYA
* * * * *
* * * * *

S. aureusPSESKEYVYIIDEVHMLTGA FNALLKTLLEPPAHAFILATTEPHKIPPTLISRA
B. sub PSAVTYKVIIDEVHMLSIGAFNALKTLLEPPHCIFILATTEPHKIPPTLISRC
E. coli PARGRFKVYLIDEVHMLSRHSFNALLKTLLEPPHVKFLATTPQKLPVTLISRC
* * * * *
* * * * *

FIGURE 10

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```
S.aureus
B.sub
E.coli
Sal.typ

S.aureus
B.sub
E.coli
Sal.typ

S.aureus
B.sub
E.coli
Sal.typ

S.aureus
B.sub
E.coli
Sal.typ
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FIGURE 11

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SEQUENCE LISTING

<110> The Rockefeller University

<120> DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND
THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS

<130> 22221/1003

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<150> 60/074,522

<151> 1998-01-27

<150> 60/093,727

<151> 1998-07-22

<160> 44

<170> PatentIn Ver. 2.0

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<213> Staphylococcus aureus

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<213> Staphylococcus aureus

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          20             25             30

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Phe Gly His Leu Leu Ala Lys Ala Val Ala Lys Asp Val Gly Arg Ile
      35             40             45

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Met Gly Phe Asp Glu Val Thr Leu Asn Glu Ile Ser Ser Leu Ile Pro
      50             55             60

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His Lys Leu Gly Ile Thr Leu Asp Glu Ala Tyr Gln Ile Asp Asp Phe
      65             70             75             80

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Lys Lys Phe Val His Arg Asn His Arg His Gln Arg Trp Phe Ser Ile
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Cys Lys Lys Leu Glu Gly Leu Pro Arg His Thr Ser Thr His Ala Ala
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Gly Ile Ile Ile Asn Asp His Pro Leu Tyr Glu Tyr Ala Pro Leu Thr
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Lys Gly Asp Thr Gly Leu Leu Thr Gln Trp Thr Met Thr Glu Ala Glu
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Ile Ile His Gln Ile Leu Thr Arg Val Glu Lys Asp Leu Gly Phe Asn
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 195 200 205

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 210 215 220

Val Ala Val Thr Ser Leu Tyr Arg Pro Gly Pro Met Glu Glu Ile Pro
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Thr Tyr Ile Thr Arg Arg His Asp Pro Ser Lys Val Gln Tyr Leu His
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Pro His Leu Glu Pro Ile Leu Lys Asn Thr Tyr Gly Val Ile Ile Tyr
 260 265 270

Gln Glu Gln Ile Met Gln Ile Ala Ser Thr Phe Ala Asn Phe Ser Tyr
 275 280 285

Gly Glu Ala Asp Ile Leu Arg Arg Ala Met Ser Lys Lys Asn Arg Ala
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Val Leu Glu Arg Asp Ala Gln His Phe Ile Glu Gly Thr Lys Gln Asn
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Gly Tyr His Glu Asp Ile Ser Lys Gln Ile Phe Asp Leu Ile Leu Lys
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 370 375 380

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Ile Asn Glu Ser His Trp Phe Tyr Lys Pro Ser Gln Glu Gly Ile Tyr
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Leu Ser Ile Gly Thr Ile Lys Gly Val Gly Tyr Gln Ser Val Lys Val
 420 425 430

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 515 520 525
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gttcgaacgg agtatattaca aaatcgtaaa aacgaaggcg atgatatgcc aaagcaacaa 1620
gcacaacaaa cagatattgc tcaaaaagca aaagatcttt tcggtgaaga aactgtacat 1680
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```

<210> 4

<211> 566

<212> PRT

<213> Staphylococcus aureus

<400> 4

```

Leu Asn Tyr Gln Ala Leu Tyr Arg Met Tyr Arg Pro Gln Ser Phe Glu
  1                      5                      10                     15

```

```

Asp Val Val Gly Gln Glu His Val Thr Lys Thr Leu Arg Asn Ala Ile
      20                      25                     30

```

```

Ser Lys Glu Lys Gln Ser His Ala Tyr Ile Phe Ser Gly Pro Arg Gly
      35                      40                     45

```

```

Thr Gly Lys Thr Ser Ile Ala Lys Val Phe Ala Lys Ala Ile Asn Cys
      50                      55                     60

```

```

Leu Asn Ser Thr Asp Gly Glu Pro Cys Asn Glu Cys His Ile Cys Lys
      65                      70                     75                     80

```

```

Gly Ile Thr Gln Gly Thr Asn Ser Asp Val Ile Glu Ile Asp Ala Ala
      85                      90                     95

```

```

Ser Asn Asn Gly Val Asp Glu Ile Arg Asn Ile Arg Asp Lys Val Lys
      100                     105                    110

```

```

Tyr Ala Pro Ser Glu Ser Lys Tyr Lys Val Tyr Ile Ile Asp Glu Val
      115                     120                    125

```

```

His Met Leu Thr Thr Gly Ala Phe Asn Ala Leu Leu Lys Thr Leu Glu

```


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130	135	140
Glu Pro Pro Ala His Ala Ile Phe Ile Leu Ala Thr Thr Glu Pro His		
145	150	155 160
Lys Ile Pro Pro Thr Ile Ile Ser Arg Ala Gln Arg Phe Asp Phe Lys		
	165	170 175
Ala Ile Ser Leu Asp Gln Ile Val Glu Arg Leu Lys Phe Val Ala Asp		
	180	185 190
Ala Gln Gln Ile Glu Cys Glu Asp Glu Ala Leu Ala Phe Ile Ala Lys		
	195	200 205
Ala Ser Glu Gly Gly Met Arg Asp Ala Leu Ser Ile Met Asp Gln Ala		
	210	215 220
Ile Ala Phe Gly Asp Gly Thr Leu Thr Leu Gln Asp Ala Leu Asn Val		
	225	230 235 240
Thr Gly Ser Val His Asp Glu Ala Leu Asp His Leu Phe Asp Asp Ile		
	245	250 255
Val Gln Gly Asp Val Gln Ala Ser Phe Lys Lys Tyr His Gln Phe Ile		
	260	265 270
Thr Glu Gly Lys Glu Val Asn Arg Leu Ile Asn Asp Met Ile Tyr Phe		
	275	280 285
Val Arg Asp Thr Ile Met Asn Lys Thr Ser Glu Lys Asp Thr Glu Tyr		
	290	295 300
Arg Ala Leu Met Asn Leu Glu Leu Asp Met Leu Tyr Gln Met Ile Asp		
	305	310 315 320
Leu Ile Asn Asp Thr Leu Val Ser Ile Arg Phe Ser Val Asn Gln Asn		
	325	330 335
Val His Phe Glu Val Leu Leu Val Lys Leu Ala Glu Gln Ile Lys Gly		
	340	345 350
Gln Pro Gln Val Ile Ala Asn Val Ala Glu Pro Ala Gln Ile Ala Ser		
	355	360 365
Ser Pro Asn Thr Asp Val Leu Leu Gln Arg Met Glu Gln Leu Glu Gln		
	370	375 380
Glu Leu Lys Thr Leu Lys Ala Gln Gly Val Ser Val Ala Pro Thr Gln		

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```

385              390              395              400
Lys Ser Ser Lys Lys Pro Ala Arg Gly Ile Gln Lys Ser Lys Asn Ala
              405              410              415
Phe Ser Met Gln Gln Ile Ala Lys Val Leu Asp Lys Ala Asn Lys Ala
              420              425              430
Asp Ile Lys Leu Leu Lys Asp His Trp Gln Glu Val Ile Asp His Ala
              435              440              445
Gln Asn Asn Asp Lys Lys Ser Leu Val Ser Leu Leu Gln Asn Ser Glu
              450              455              460
Pro Val Ala Ala Ser Glu Asp His Val Leu Val Lys Phe Glu Glu Glu
465              470              475              480
Ile His Cys Glu Ile Val Asn Lys Asp Asp Glu Lys Arg Ser Ser Ile
              485              490              495
Glu Ser Val Val Cys Asn Ile Val Asn Lys Asn Val Lys Val Val Gly
              500              505              510
Val Pro Ser Asp Gln Trp Gln Arg Val Arg Thr Glu Tyr Leu Gln Asn
              515              520              525
Arg Lys Asn Glu Gly Asp Asp Met Pro Lys Gln Gln Ala Gln Gln Thr
              530              535              540
Asp Ile Ala Gln Lys Ala Lys Asp Leu Phe Gly Glu Glu Thr Val His
545              550              555              560
Val Ile Asp Glu Glu Glx
              565

```

<210> 5

<211> 2034

<212> DNA

<213> Staphylococcus aureus

<400> 5

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gataataaag aaattgatgt tgtaacattg atggatcaat tatcgacgga aggtacgttg 240
aatgaagcgg gtggcccgca atatcttgca gagttatcta caaatgtacc aacgacgcga 300
aatgttcagt attatactga tatcgtttct aagcatgcat taaaacgtag attgattcaa 360

```

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```

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gacattcgag acgtcttagg acaagtgtat gaaacagctg aagagcttga tcaaaatagt 540
gggtcaaacac caggtatacc tacaggatat cgagatttag accaaatgac agcagggttc 600
aaccgaaaatg atttaattat ccttgacgag cgtccatctg taggtaagac tgcgttcgca 660
cttaatatgg cacaataaagt tgcaacgcat gaagatatgt atacagttaa aagcaacagg 720
aagtttctga aatctctcgt acattaaaag cattagcccg tgaattaaaa tgtccagtta 780
tcgcattaag tcagttatct cgtggtgttg aacaacgaca agataaacgt ccaatgatga 840
gtgatattcg tgaatctggt tcgattgagc aagatgccga tatcgttgca ttcttatacc 900
gtgatgatta ctataaccgt ggcggcgatg aagatgatga cgatgatggt ggtttcgagc 960
cacaacagaa tgatgaaaac ggtgaaattg aaattatcat tgtaagcaa cgtaacggtc 1020
caacaggcac agttaagtta ctttttatga aacaatataa taaattttag agctatcatc 1080
ttttcttgaa agcgatggct ttaaagacat tcgagacgtc ttaggacaag tgtatgaaac 1140
agctgaagag cttgatcaaa atagtgtgca aacaccaggt atacctacag gatatcgaga 1200
tttagacca atgacagcag gggtcaaccg aaatgattta attatccttg cagcgcgctc 1260
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ctagagatgg gtgctgatca gtttaaccaca cgtatgattt gtagttctgg taatgttgac 1440
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gttggtaaat tatcacgtac gaagattttt attgatgata caccgggtat tcgaattaat 1560
gatttacgtt ctaaatgtcg tcgattaaag caagaacatg gcttagacat gattgtgatt 1620
gactacttac agttgattca aggtagtgtt tcacgtgctg ccgataacag acaacaggaa 1680
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gcattaagtc agttatctcg tgggtgtgaa caacgacaag ataaacgtcc aatgatgagt 1800
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gatgattact ataaccgtgg cggcgatgaa gatgatgacg atgatgggtg ttcgagcccc 1920
caaacgaatg atgaaaacgg tgaaattgaa attatcattg ctaagcaacg ttacgggtcca 1980
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```

<210> 6

<211> 457

<212> PRT

<213> Staphylococcus aureus

<400> 6

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Met Asp Arg Met Tyr Glu Gln Asn Gln Met Pro His Asn Asn Glu Ala
  1              5              10              15

```

```

Glu Gln Ser Val Leu Gly Ser Ile Ile Ile Asp Pro Glu Leu Ile Asn
      20              25              30

```

```

Thr Thr Gln Glu Val Leu Leu Pro Glu Ser Phe Tyr Arg Gly Ala His
    35              40              45

```

```

Gln His Ile Phe Arg Ala Met Met His Leu Asn Glu Asp Asn Lys Glu
    50              55              60

```

```

Ile Asp Val Val Thr Leu Met Asp Gln Leu Ser Thr Glu Gly Thr Leu

```

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65	70	75	80
Asn Glu Ala Gly Gly Pro Gln Tyr Leu Ala Glu Leu Ser Thr Asn Val	85	90	95
Pro Thr Thr Arg Asn Val Gln Tyr Tyr Thr Asp Ile Val Ser Lys His	100	105	110
Ala Leu Lys Arg Arg Leu Ile Gln Thr Ala Asp Ser Ile Ala Asn Asp	115	120	125
Gly Tyr Asn Asp Glu Leu Glu Leu Asp Ala Ile Leu Ser Asp Ala Glu	130	135	140
Arg Arg Ile Leu Glu Leu Ser Ser Ser Arg Glu Ser Asp Gly Phe Lys	145	150	155
Asp Ile Arg Asp Val Leu Gly Gln Val Tyr Glu Thr Ala Glu Glu Leu	165	170	175
Asp Gln Asn Ser Gly Gln Thr Pro Gly Ile Pro Thr Gly Tyr Arg Asp	180	185	190
Leu Asp Gln Met Thr Ala Gly Phe Asn Arg Asn Asp Leu Ile Ile Leu	195	200	205
Ala Ala Arg Pro Ser Val Gly Lys Thr Ala Phe Ala Leu Asn Ile Ala	210	215	220
Gln Lys Leu Glu Arg Met Lys Ile Tyr Leu Ala Val Gly Ile Phe Ser	225	230	235
Leu Glu Met Gly Ala Asp Gln Leu Thr Thr Arg Met Ile Cys Ser Ser	245	250	255
Gly Asn Val Asp Ser Asn Arg Leu Arg Thr Gly Thr Met Thr Glu Glu	260	265	270
Asp Trp Ser Arg Phe Thr Ile Ala Val Gly Lys Leu Ser Arg Thr Lys	275	280	285
Ile Phe Ile Asp Asp Thr Pro Gly Ile Arg Ile Asn Asp Leu Arg Ser	290	295	300
Lys Cys Arg Arg Leu Lys Gln Glu His Gly Leu Asp Met Ile Val Ile	305	310	315
Asp Tyr Leu Gln Leu Ile Gln Gly Ser Gly Ser Arg Ala Ser Asp Asn			

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```

          325              330              335
Arg Gln Gln Glu Val Ser Glu Ile Ser Arg Thr Leu Lys Ala Leu Ala
          340              345              350
Arg Glu Leu Lys Cys Pro Val Ile Ala Leu Ser Gln Leu Ser Arg Gly
          355              360              365
Val Glu Gln Arg Gln Asp Lys Arg Pro Met Met Ser Asp Ile Arg Glu
          370              375              380
Ser Gly Ser Ile Glu Gln Asp Ala Asp Ile Val Ala Phe Leu Tyr Arg
          385              390              395              400
Asp Asp Tyr Tyr Asn Arg Gly Gly Asp Glu Asp Asp Asp Asp Asp Gly
          405              410              415
Gly Phe Glu Pro Gln Thr Asn Asp Glu Asn Gly Glu Ile Glu Ile Ile
          420              425              430
Ile Ala Lys Gln Arg Tyr Gly Pro Gly Thr Val Lys Leu Leu Phe Met
          435              440              445
Lys Gln Tyr Gly Lys Phe Thr Asp Ile
          450              455

```

<210> 7

<211> 4266

<212> DNA

<213> Staphylococcus aureus

<400> 7

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acatgggaat ttcattattac attaccacaa ttcttagctc atgaagatta tttattatit 180
ataaatgcaa tagagcaaga gtttaaagat atcgccaacg ttacatgtcg ttttacggt 240
acaaatggca cgaatcaaga tgaacatgca attaaatact ttgggcactg tattgaccaa 300
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aaagtattaa aagtaatggt atcaaatgac attgaacgta atcattttga taaggcatgt 420
aatggaagtc ttatcaaagc gtttagaaat tgtggttttg atatcgataa aatcatattc 480
gaaacaaatg ataatgatca agaacaaaac ttagctttct tagaagcaca tattcaagaa 540
gaagacgaac aaagtgcacg attggcaaca gagaaacttg aaaaaatgaa agctgaaaaa 600
gcgaaacaac aagataacaa gcaaagtgtc gtcgataagt gtcaaattgg taagccgatt 660
caaattgaaa atattaaacc aattgaatct attattgagg aagagtttaa agttgcaata 720
gagggtgtca tttttgatat aaacttaaaa gaacttaaaa gtggtcgcca tatcgtagaa 780
attaaagtga ctgactatac ggactcttta gttttaaaaa tgtttactcg taaaaacaaa 840
gatgatttag aacattttta agcgctaagt gttggtaa at gggttagggc tcaaggctcg 900

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```

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gcaatgagcc aaatggatgg tataccaaat attgggtcgt atgttaaaca ggcagcagac 1080
tggggacatc cagccattgc gggtacagac cataatgtgg tgcaagcatt tccagatgct 1140
cacgcagcag cggaaaaaca tggcattaaa atgatatacg gtatggaagg tatgttagtt 1200
gatgatgggtg ttccgattgc atacaaacca caagatgtcg tattaaaaga tgcctacttat 1260
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catgaacgat tatcggaaac gattatcaat ttgacgcata ttactgatga tatgttagta 1440
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gaaatgattg aaacgatgaa agaaaatgaa gtgccagatt ggtattttaga ttcattgctt 3720
aaaattaagt acatattccc taaagcccat gcagcagcat acgttttaat ggcagtagct 3780

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atcgcatatt tcaaagtaca tcattccatt tattactatg catcttactt tacaattcgt 3840
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atgtaa                                     4266

```

<210> 8

<211> 1435

<212> PRT

<213> Staphylococcus aureus

<400> 8

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Met Thr Glu Gln Gln Lys Phe Lys Val Leu Ala Asp Gln Ile Lys Ile
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Ser Asn Gln Leu Asp Ala Glu Ile Leu Asn Ser Gly Glu Leu Thr Arg
          20              25              30

Ile Asp Val Ser Asn Lys Asn Arg Thr Trp Glu Phe His Ile Thr Leu
      35              40              45

Pro Gln Phe Leu Ala His Glu Asp Tyr Leu Leu Phe Ile Asn Ala Ile
      50              55              60

Glu Gln Glu Phe Lys Asp Ile Ala Asn Val Thr Cys Arg Phe Thr Val
      65              70              75              80

Thr Asn Gly Thr Asn Gln Asp Glu His Ala Ile Lys Tyr Phe Gly His
          85              90              95

Cys Ile Asp Gln Thr Ala Leu Ser Pro Lys Val Lys Gly Gln Leu Lys
      100              105              110

Gln Lys Lys Leu Ile Met Ser Gly Lys Val Leu Lys Val Met Val Ser
      115              120              125

Asn Asp Ile Glu Arg Asn His Phe Asp Lys Ala Cys Asn Gly Ser Leu
      130              135              140

Ile Lys Ala Phe Arg Asn Cys Gly Phe Asp Ile Asp Lys Ile Ile Phe
      145              150              155              160

Glu Thr Asn Asp Asn Asp Gln Glu Gln Asn Leu Ala Ser Leu Glu Ala
          165              170              175

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His Ile Gln Glu Glu Asp Glu Gln Ser Ala Arg Leu Ala Thr Glu Lys
180 185 190

Leu Glu Lys Met Lys Ala Glu Lys Ala Lys Gln Gln Asp Asn Lys Gln
195 200 205

Ser Ala Val Asp Lys Cys Gln Ile Gly Lys Pro Ile Gln Ile Glu Asn
210 215 220

Ile Lys Pro Ile Glu Ser Ile Ile Glu Glu Glu Phe Lys Val Ala Ile
225 230 235 240

Glu Gly Val Ile Phe Asp Ile Asn Leu Lys Glu Leu Lys Ser Gly Arg
245 250 255

His Ile Val Glu Ile Lys Val Thr Asp Tyr Thr Asp Ser Leu Val Leu
260 265 270

Lys Met Phe Thr Arg Lys Asn Lys Asp Asp Leu Glu His Phe Lys Ala
275 280 285

Leu Ser Val Gly Lys Trp Val Arg Ala Gln Gly Arg Ile Glu Glu Asp
290 295 300

Thr Phe Ile Arg Asp Leu Val Met Met Met Ser Asp Ile Glu Glu Ile
305 310 315 320

Lys Lys Ala Thr Lys Lys Asp Lys Ala Glu Glu Lys Arg Val Glu Phe
325 330 335

His Leu His Thr Ala Met Ser Gln Met Asp Gly Ile Pro Asn Ile Gly
340 345 350

Ala Tyr Val Lys Gln Ala Ala Asp Trp Gly His Pro Ala Ile Ala Val
355 360 365

Thr Asp His Asn Val Val Gln Ala Phe Pro Asp Ala His Ala Ala Ala
370 375 380

Glu Lys His Gly Ile Lys Met Ile Tyr Gly Met Glu Gly Met Leu Val
385 390 395 400

Asp Asp Gly Val Pro Ile Ala Tyr Lys Pro Gln Asp Val Val Leu Lys
405 410 415

Asp Ala Thr Tyr Val Val Phe Asp Val Glu Thr Thr Gly Leu Ser Asn
420 425 430

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Gln Tyr Asp Lys Ile Ile Glu Leu Ala Ala Val Lys Val His Asn Gly
435 440 445

Glu Ile Ile Asp Lys Phe Glu Arg Phe Ser Asn Pro His Glu Arg Leu
450 455 460

Ser Glu Thr Ile Ile Asn Leu Thr His Ile Thr Asp Asp Met Leu Val
465 470 475 480

Asp Ala Pro Glu Ile Glu Glu Val Leu Thr Glu Phe Lys Glu Trp Val
485 490 495

Gly Asp Ala Ile Phe Val Ala His Asn Ala Ser Phe Asp Met Gly Phe
500 505 510

Ile Asp Thr Gly Tyr Glu Arg Leu Gly Phe Gly Pro Ser Thr Asn Gly
515 520 525

Val Ile Asp Thr Leu Glu Leu Ser Arg Thr Ile Asn Thr Glu Tyr Gly
530 535 540

Lys His Gly Leu Asn Phe Leu Ala Lys Lys Tyr Gly Val Glu Leu Thr
545 550 555 560

Gln His His Arg Ala Ile Tyr Asp Thr Glu Ala Thr Ala Tyr Ile Phe
565 570 575

Ile Lys Met Val Gln Gln Met Lys Glu Leu Gly Val Leu Asn His Asn
580 585 590

Glu Ile Asn Lys Lys Leu Ser Asn Glu Asp Ala Tyr Lys Arg Ala Arg
595 600 605

Pro Ser His Val Thr Leu Ile Val Gln Asn Gln Gln Gly Leu Lys Asn
610 615 620

Leu Phe Lys Ile Val Ser Ala Ser Leu Val Lys Tyr Phe Tyr Arg Thr
625 630 635 640

Pro Arg Ile Pro Arg Ser Leu Leu Asp Glu Tyr Arg Glu Gly Leu Leu
645 650 655

Val Gly Thr Ala Cys Asp Glu Gly Glu Leu Phe Thr Ala Val Met Gln
660 665 670

Lys Asp Gln Ser Gln Val Glu Lys Ile Ala Lys Tyr Tyr Asp Phe Ile
675 680 685

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Glu Ile Gln Pro Pro Ala Leu Tyr Gln Asp Leu Ile Asp Arg Glu Leu
690 695 700

Ile Arg Asp Thr Glu Thr Leu His Glu Ile Tyr Gln Arg Leu Ile His
705 710 715 720

Ala Gly Asp Thr Ala Gly Ile Pro Val Ile Ala Thr Gly Asn Ala His
725 730 735

Tyr Leu Phe Glu His Asp Gly Ile Ala Arg Lys Ile Leu Ile Ala Ser
740 745 750

Gln Pro Gly Asn Pro Leu Asn Arg Ser Thr Leu Pro Glu Ala His Phe
755 760 765

Arg Thr Thr Asp Glu Met Leu Asn Glu Phe His Phe Leu Gly Glu Glu
770 775 780

Lys Ala His Glu Ile Val Val Lys Asn Thr Asn Glu Leu Ala Asp Arg
785 790 795 800

Ile Glu Arg Val Val Pro Ile Lys Asp Glu Leu Tyr Thr Pro Arg Met
805 810 815

Glu Gly Ala Asn Glu Glu Ile Arg Glu Leu Ser Tyr Ala Asn Ala Arg
820 825 830

Lys Leu Tyr Gly Glu Asp Leu Pro Gln Ile Val Ile Asp Arg Leu Glu
835 840 845

Lys Glu Leu Lys Ser Ile Ile Gly Asn Gly Phe Ala Val Ile Tyr Leu
850 855 860

Ile Ser Gln Arg Leu Val Lys Lys Ser Leu Asp Asp Gly Tyr Leu Val
865 870 875 880

Gly Ser Arg Gly Ser Val Gly Ser Ser Phe Val Ala Thr Met Thr Glu
885 890 895

Ile Thr Glu Val Asn Pro Leu Pro Pro His Tyr Ile Cys Pro Asn Cys
900 905 910

Lys Thr Ser Glu Phe Phe Asn Asp Gly Ser Val Gly Ser Gly Phe Asp
915 920 925

Leu Pro Asp Lys Thr Cys Glu Thr Cys Gly Ala Pro Leu Ile Lys Glu
930 935 940

PCT/US99/01547

Gln Glu Leu Ile Lys Thr Gly Ile Cys Asp Leu Ser Ser Val Ile Gly
1185 1190 1195 1200

PCT/US99/01547

PCT/US99/01547

<213> Staphylococcus aureus

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<213> Staphylococcus aureus

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Ser Pro Glu Ile Gly Thr Val Lys Glu Glu Val Asp Ala Asn Asp Val
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gctgtagata ttgaggcaac acaatctaac tcaaatgttc aaattgcttc tgatgattta 240
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 35 40 45

Glu Ala Val Lys Glu Leu Gly Asp Arg Val Asn Val Ala Val Asp Ile
 50 55 60

Glu Ala Thr Gln Ser Asn Ser Asn Val Gln Ile Ala Ser Asp Asp Leu
 65 70 75 80

Gln Met Ile Glu Met His Glu Leu Ile Gln Glu Phe Tyr Tyr Tyr Ala
 85 90 95

Leu Thr Lys Thr Val Glu Gly Glu Gln Ala Leu Thr Tyr Leu Gln Glu
 100 105 110

Arg Gly Phe Thr Asp Ala Leu Ile Lys Glu Arg Gly Ile Gly Phe Ala
 115 120 125

Pro Asp Ser Ser His Phe Cys His Asp Phe Leu Gln Lys Lys Gly Tyr
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Asn Phe Ser Tyr Tyr Asp Arg Phe Arg Asn Arg Ile Met Phe Pro Leu
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Lys Asn Ala Gln Gly Arg Ile Val Gly Tyr Ser Gly Arg Thr Tyr Thr
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Gly Gln Glu Pro Lys Tyr Leu Asn Ser Pro Glu Thr Pro Ile Phe Gln
 195 200 205

Lys Arg Lys Leu Leu Tyr Asn Leu Asp Lys Ala Arg Lys Ser Ile Arg
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Lys Leu Asp Glu Ile Val Leu Leu Glu Gly Phe Met Asp Val Ile Lys
 225 230 235 240

Ser Asp Thr Ala Gly Leu Lys Asn Val Val Ala Thr Met Gly Thr Gln

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Gly Gly Tyr Ile Glu Pro Glu Pro Ile Gly Met Ala Gln Phe Asp Asn		
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500

505

510

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41

48

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48

31

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01547

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : C07H 21/04, C12N 15/11, 15/63, 1/20, 9/00 US CL : 536/23.1, 435/320.1, 435/252.1+, 435 /183+ According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/23.1, 435/320.1, 435/252.1+, 435 /183+ Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)														
C. DOCUMENTS CONSIDERED TO BE RELEVANT														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X - Y	KUNST et al. The complete genome sequence of the Gram-positive bacterium <i>Bacillus subtilis</i> . <i>Nature</i> . 20 November 1997, Vol. 390, pages 249-256, see entire article.	1 ---- 2-3, 6-9												
X - Y	SAUER et al. Sporulation and Primary Sigma Factor Homologous Genes in <i>Clostridium acetobutylicum</i> . <i>J Bacteriol.</i> November 1994, Vol. 176, No. 21, pages 6572-6582, see entire article.	1 ---- 2-3, 6-9												
Y	FRASER et al. The Minimal Gene Complement of <i>Mycoplasma genitalium</i> . <i>Science</i> . 20 October 1995, Vol. 270, pages 397-403, see entire document.	1-3, 6-9												
X	US 5,151,350 A (COLBERT et al) 29 September 1992 (29/09/92), examples 1-7.	1-9												
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>*A* document defining the general state of the art which is not considered to be of particular relevance</td> <td>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>*E* earlier document published on or after the international filing date</td> <td>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>*G* document member of the same patent family</td> </tr> <tr> <td>*O* document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>*P* document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family	*O* document referring to an oral disclosure, use, exhibition or other means		*P* document published prior to the international filing date but later than the priority date claimed	
* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention													
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone													
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art													
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family													
O document referring to an oral disclosure, use, exhibition or other means														
P document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search 10 MAY 1999		Date of mailing of the international search report 20 MAY 1999												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer <i>[Signature]</i> Lee, Li Telephone No. (703) 308-0196												

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01547

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,583,026 A (O'DONNELL) 10 December 1996 (10/12/96), Summary of the Invention.	1-5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US99/01547

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US99/01547

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

- Group I, claims 1-9, drawn to DNA molecule corresponding to dnaE and expression system.
- Group II, claims 10-13, drawn to dnaE protein.
- Group III, claims 14-22, drawn to DNA molecule corresponding to dnaX and expression system.
- Group IV, claims 23-26, drawn to dnaX protein.
- Group V, claims 27-35, drawn to DNA molecule corresponding to dnaB and expression system.
- Group VI, claims 36-39, drawn to dnaB protein.
- Group VII, claims 40, 50-53, 59-60, drawn to method of identifying compounds which inhibit activity of a DNA polymerase per se.
- Group VIII, claims 41- 42, 59-60, drawn to method of identifying compounds which inhibit the ability of a beta subunit to stimulate a DNA polymerase.
- Group IX, claims 43- 44, 59-60, drawn to method of identifying compounds which inhibit the ability of a beta subunit and a DNA polymerase to interact physically.
- Group X, claims 45, 59-60, drawn to method of identifying compounds which inhibit the ability of a subunit to assemble a beta subunit on a DNA molecule.
- Group XI, claims 46 - 47, 59-60, drawn to method of identifying compounds which inhibit the ability of a subunit to disassemble a beta subunit from a DNA molecule.
- Group XII, claims 48- 49, 59-60, drawn to method of identifying compounds which inhibit dATP/ATP binding activity of a gamma complex.
- Group XIII, claims 54, 59-60, drawn to method of identifying compounds which inhibit a DnaB helicase.
- Group XIV, claims 55, 59-60, drawn to method of identifying compounds which inhibit nucleoside triphosphate activity of a DnaB helicase.
- Group XV, claims 56, 59-60, drawn to method of identifying compounds which inhibit primase activity.
- Group XVI, claims 57, 59-60, drawn to method of identifying compounds which inhibit the ability of a primase and a DnaB protein to interact.
- Group XVII, claims 58, 59-60, drawn to method of identifying compounds which inhibit the ability of a DNA molecule and a DnaB protein to interact.

The inventions listed as Groups I-XVII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I invention of a nucleotide sequence of dnaE. The special technical feature of the Group I invention of the nucleotide sequence is not present in the Group II invention of the particular isolated protein dnaE from a Gram positive bacterium and the protein of SEQ. ID. No. 2.

The special technical feature of the Group III invention of a nucleotide sequence of dnaX. The special technical feature of the Group III invention of the nucleotide sequence is not present in the Group IV invention of the particular isolated protein dnaX from a Gram positive bacterium and the protein of SEQ. ID. No. 4.

The special technical feature of the Group V invention of a nucleotide sequence of dnaB. The special technical feature of the Group V invention of the nucleotide sequence is not present in the Group VI invention of the particular isolated protein dnaB from a Gram positive bacterium and the protein of SEQ. ID. No. 6.

Group I, III and V have distinct nucleotide sequences (e.g., SEQ. ID. No.1, SEQ. ID. No.3, and SEQ. ID. No.5) encode different proteins which have different structures and biological properties while Group II, IV and VI have distinct amino acid sequences (e.g., ID. No.2, SEQ. ID. No.4, and SEQ. ID. No.6) which are different proteins having different structure and biological properties. Thus, the inventions Groups (I, III, V) and Groups (II, IV, VI,) have distinct technical features from each other and the distinct technical features are not present in each other's inventions.

Groups VII and (VIII - XVII) have distinct special technical features of particular methods from each other and the distinct special technical features particular methods are not present in each other's inventions. Those particular methods of identifying compounds have different method objective, different method steps and different reagents used. For example, the special technical feature in Group VII is particular method of identifying compounds which inhibit activity of a DNA polymerase per se, the special technical feature in Group VIII is particular method of identifying compounds which inhibit the ability of a beta subunit to stimulate a DNA polymerase, the special technical feature in Group IX is particular method of identifying compounds which inhibit the ability of a beta subunit and a DNA

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polymerase to interact physically, the special technical feature in Group X is particular method of identifying compounds which inhibit the ability of a subunit to assemble a beta subunit on a DNA molecule, the special technical feature in Group XI is particular method of identifying compounds which inhibit the ability of a subunit to disassemble a beta subunit from a DNA molecule, the special technical feature in Group XII is particular method of identifying compounds which inhibit dATP/ATP binding activity of a gamma complex, the special technical feature in Group XIII is particular method of identifying compounds which inhibit a DnaB helicase, the special technical feature in Group XIV is particular method of identifying compounds which inhibit nucleoside triphosphate activity of a DnaB helicase, the special technical feature in Group XV is particular method of identifying compounds which inhibit primase activity, the special technical feature in Group XVI is particular method of identifying compounds which inhibit the ability of a primase and a DnaB protein to interact, and the special technical feature in Group XVII is particular method of identifying compounds which inhibit the ability of a DNA molecule and a DnaB protein to interact.

Since the special technical features of particular nucleotide sequences and nucleotide hybridizing (e.g., SEQ. ID. No.1, SEQ. ID. No.3, and SEQ. ID. No.5) and protein sequences (e.g., SEQ. ID. No.2, SEQ. ID. No.4, SEQ. ID. No.6) in inventions of Groups I-VI are not required in inventions of Groups VII-XVII of methods of identifying compounds, Groups I-VI and VII-XVII lack unity with each other.